Physiological Mini Reviews

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SCHCF + ALACF 2020 joint meeting
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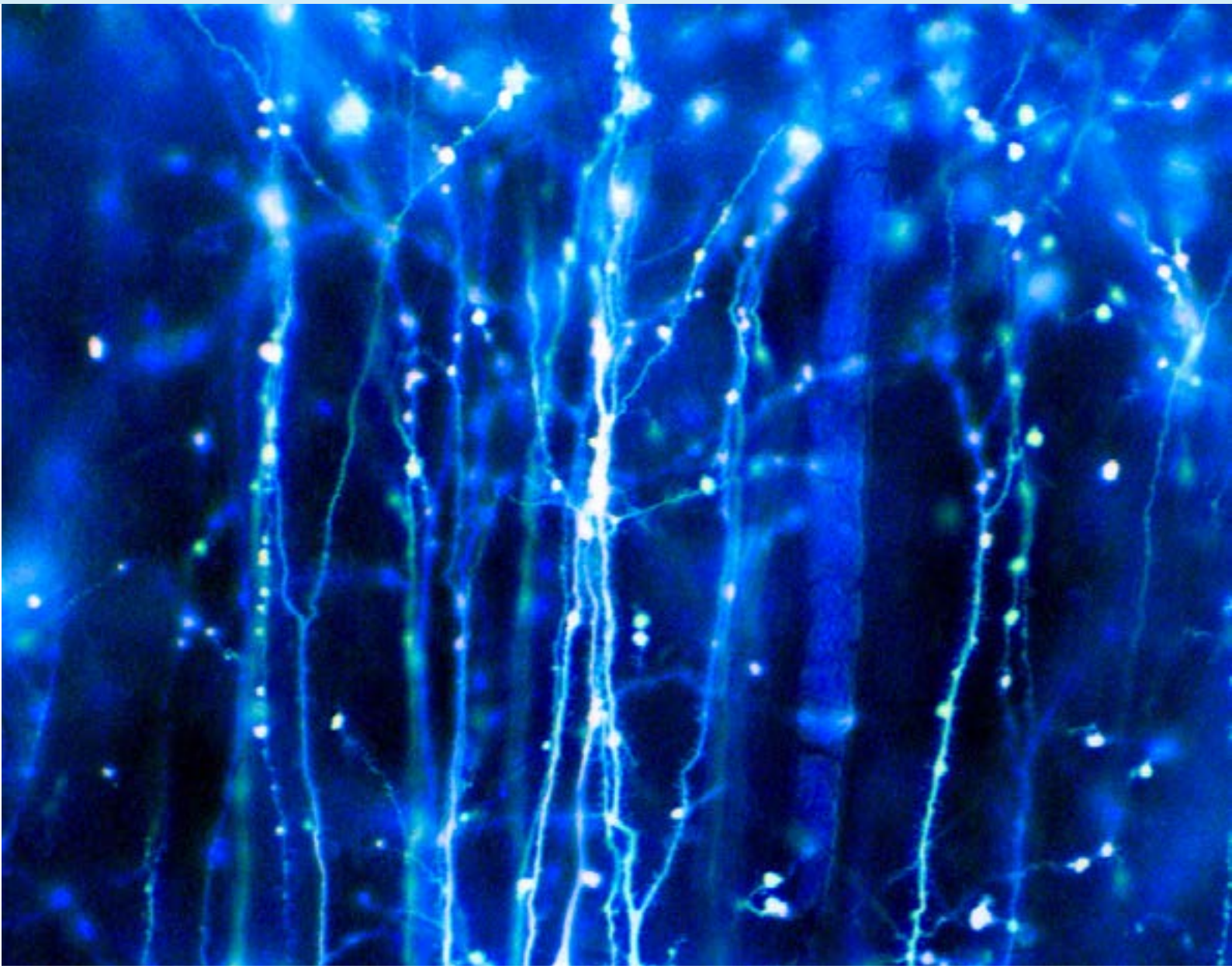

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Physiological Mini Reviews

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Physiological Mini-Reviews is a scientific journal, publishing brief reviews on "hot" topics in Physiology. The scope is quite broad, going from "Molecular Physiology" to "Integrated Physiological Systems". As indicated by our title it is not our intention to publish exhaustive and complete reviews. We ask to the authors concise and updated descriptions of the "state of the art" in a specific topic. Innovative and thought-provoking ideas are welcome.

Preparation and Submission of manuscripts:

"Physiological Mini-Reviews" will have a maximum of 3000 words, 50 references and 3 figures. Material will be addressed to scientific people in general but not restricted to specialist of the field. For citations in the text please refer to Instructions in our webpage. Final format will be given at the Editorial Office. Most contributions will be invited ones, but spontaneous presentations are welcome. Send your manuscript in Word format (.doc or .docx) to: pmr@safisiol.org.ar

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PROGRAM & ABSTRACTS

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Programme-at-glance – SCHCF+ALACF 2020 joint meeting – November 16-20, 2020 (virtual)

	Monday 16	Tuesday 17	Wednesday 18	Thursday 19	Friday 20
9:00-10:00	**Opening L Sobrevia** (SCHCF & ALACF) **Inaugural lecture** **J Chan** (Taiwan, IUPS) *Nitric oxide in the control of blood pressure: the good, the bad and the ugly*	**S3** – The exposome and metabolic diseases	**S7** – New approaches to identify oncogenic transformation across cancer progression – focus on extracellular vesicles	**S10** – Physiology of extracellular vesicles in cardiovascular disease	**S14** – Stem cells in regenerative medicine: targeted diseases
10:15-11:15	**S1** – New advances in cardiorespiratory neural control	**S4** – Preeclampsia more than hypertension in pregnancy	**S8** – T-tubules as regulators of heart rhythm and function	**S11** – TRP channels: health and disease	**S15** – The social environment and the physiological state: what changes depending on who is around? Experiences in ruminants
11:30-12:30	**L1 – M Moraes** (Brazil) *Physiopathology of epilepsy: from things working to going terribly wrong*	**L3 – S Deuchars** (UK, TPS) *Non-invasive neuromodulation of the autonomic nervous system*	**L5 – H Trimarchi** (Argentina) *The podocyte. New concepts*	**L8 – D Mikhailidis** (UK) *Treating hypercholesterolaemia: an update with a focus on evolocumab*	**L10 – JC Calderón** (Colombia) *The role of skeletal muscle in the pathophysiology of the metabolic syndrome: muscle mass, fiber types and myokines*
14:00-15:00	**YRS1** – Aquaporins in physiological and pathological conditions	**YRS2** – Biology of exercise in metabolic disorders	**YRS3** – Protein and membrane interaction, always a good interaction? Osmoionic imbalances and signalling mechanisms in pathological contexts	**YRS4** – Sarco-endoplasmic reticulum-mitochondrial coupling in physiology and pathophysiology	**YRS5** – Inflammation in the cardiovascular system: a multifaceted pathway
15:15-16:15	**S2** – Muscle as endocrine and paracrine organs	**S5** – Physiology of kidney disease	**S9** – Molecular and cellular mechanisms in cardiac diseases	**S12** – Women in neuroinflammation: A multidisciplinary glimpse from the molecules to the translational medicine	**S16** – Lung cancer: pathophysiological aspects and advances in its treatment. The path from the molecular to the clinical
16:30-17:30	**L2 – H León-Ariza** (Colombia) *What is new in the crosstalk between the immune and nervous systems?*	**L4 – AD Mottino** (Argentina) *Regulation of the intestinal barrier associated with the organic anion-transporter MRP2 by intraluminal nutrients. Role of enteroglucagon GLP-2*	**L6 – A Myatt** (USA) *How to evaluate mobile apps in physiology and health sciences?* "All that glitters is not gold"	**L9 – L Samuelson** (USA, APS) *Niche regulation of intestinal stem cell function*	**Closing lecture** **D Eisner** (UK) *Control of calcium in the heart: free and beyond* **Closing MC Larocca** (SAFIS) *SAFIS + ALACF 2021*
17:45-18:45	**CA1 – STALab+ workshop** (Chile) *Portable laboratories for hybrid teaching in physiology*	**S6** – Endothelial function, metabolism, and signalling	**L7 – D Botero** (Colombia) *Cerebral blood flow regulation and cerebral hypoperfusion diagnosis in real time using artificial intelligence*	**S13** – Physiological approach to the type 2 diabetes mellitus treatment	
19:00-20:00		**COA – C Pérez-Leighton** (Chile) Why do I like to eat and other questions about eating and obesity	**ALACF** *General assembly*	**SCHCF** *General assembly*	

S, symposium; L, lecture; YRS, young researcher symposium; COA, community outreach activity; CA, company activity; IUPS, International Union of Physiological Sciences; ALACF, Asociación Latinoamericana de Ciencias Fisiológicas; TPS, The Physiological Society; APS, American Physiological Society; SAFIS, Sociedad Argentina de Fisiología; SCHCF, Sociedad Chilena de Ciencias Fisiológicas. Time is for Santiago de Chile (GMT-3).

LECTURES

INAUGURAL LECTURE
Julie Chan (President of IUPS, Taiwan)
Nitric oxide in the control of blood pressure: the good, the bad and the ugly

CLOSING LECTURE
David Eisner (University of Manchester & Editor of J Gen Physiol, UK)
Control of calcium in the heart: free and beyond

L1 – Márcio Moraes (Federal University of Minas Gerais, Brazil)
Physiopathology of epilepsy: from things working to going terribly wrong

L2 – Henry H León-Ariza (Universidad de La Sabana, Colombia)
What is new in the crosstalk between the immune and nervous systems?

L3 - Susan Deuchars (University of Leeds & The Physiological Society (TPS), UK)
Non-invasive neuromodulation of the autonomic nervous system

L4 – Aldo D Mottino (Universidad Nacional de Rosario, Investigador Superior CONICET, Argentina)
Regulation of the intestinal barrier associated with the organic anion-transporter MRP2 by intraluminal nutrients. Role of enteroglucagon GLP-2

L5 – Hernán Trimarchi (Hospital Británico de Buenos Aires, Argentina)
The podocyte. New concepts

L6 – Angela Myatt (USA)
How to evaluate mobile apps in physiology and health sciences? All that glitters is not gold

L7 – Daniel Botero Rosas (Universidad de La Sabana, Colombia)
Cerebral blood flow regulation and cerebral hypoperfusion diagnosis in real time using artificial intelligence

L8 – Dimitri Mikhailidis (University College London & Editor of Curr Vasc Pharmacol, UK)
Treating hypercholesterolaemia: an update with a focus on evolocumab

L9 – Linda Samuelson (University of Michigan & American Physiological Society, USA)
Niche regulation of intestinal stem cell function

L10 – Juan C Calderón (Universidad de Antioquia, Colombia)
The role of skeletal muscle in the pathophysiology of the metabolic syndrome: muscle mass, fiber types and myokines

LECTURE ABSTRACTS

INAUGURAL LECTURE

Julie Chan (President of IUPS, Taiwan)

Nitric oxide in the control of blood pressure: the good, the bad and the ugly

Manifests of body functions represent the outcomes of events that are integrated at multiple levels of systems, organs, tissues, cells and molecules. When these multilevel integrations are executed in "good" rapport, our body functions are operated in the "physiological" zone. "Pathophysiological" conditions will be instigated when they turn into "bad" relationships, leading to disease development. The "ugly" scenario will emerge on break down of the multilevel integration system, which prompts "pathological" states that heads for mortality. One illustrative example of the good, bad and ugly aspects of the multilevel integration system in the operation of body functions is the control of blood pressure (BP). Maintenance of a stable BP requires integration at the level of systems (neural, hormonal, humoral and immune systems); organs (heart, blood vessel, kidney and brain); cells (endothelial cells, smooth muscle cells, neurons, immune cells and perivascular adipocytes); and an array of molecules, including at least nitric oxide, angiotensin II, and superoxide of the reactive oxygen species. Using neural regulation of BP, particularly the role of the rostral ventrolateral medulla in the control of sympathetic vasomotor activity in health and disease as examples, the good, bad and ugly roles of NO in the integrative system for the control of blood pressure will be highlighted in this presentation. Work presented in this talk is supported by research grants OMRPG8C0051 from Chang Gung Medical Foundation, Taiwan and MOST108-2923-B-182A-MY3 from the Ministry of Science and Technology, Taiwan to JYHC.

CLOSING LECTURE

David Eisner (University of Manchester & Editor of J Gen Physiol, UK)

Control of calcium in the heart: free and beyond

I will begin the presentation by reviewing how calcium is used to regulate cell function. Since calcium cannot be destroyed, regulation of its concentration requires pumping across membranes. Importantly, on each heartbeat, the amount of calcium that enters the cell must exactly equal that which leaves. The lecture will discuss the consequences for cardiac function. 99% of the calcium in the cytoplasm is bound to buffers. It is often not appreciated that the magnitude of the change of free calcium concentration depends as much on the properties of the calcium buffers as on the underlying fluxes. Most of the Ca that activates contraction is derived from the sarcoplasmic reticulum (SR) and is released by the process of Ca induced Ca release (CICR) through the Ryanodine Receptor (RyR). On this mechanism, Ca enters the cell via the L-type Ca current and binds to the RyR making it open thereby resulting in the release of a much larger amount of Ca from the SR. The amount of Ca released depends on many factors including the properties of the RyR and the Ca content of the SR. I will discuss both the control of SR Ca content and the relationship between SR Ca content and the amplitude of the Ca transient. It is also important that during diastole, Ca is lowered to levels sufficiently low that the heart can relax and refill with blood. The mechanisms that control diastolic Ca are, however, much less well understood. I will present data suggesting that diastolic Ca is indirectly controlled by the level of systolic Ca. Specifically, reducing the systolic Ca release will decrease Ca efflux from the cell thereby tending to increase diastolic Ca. This may explain why making the RyR leaky or inhibiting SR Ca-ATPase activity elevate diastolic while decreasing systolic Ca.

L1 – Márcio Moraes (Federal University of Minas Gerais, Brazil)
Physiopathology of epilepsy: from things working to going terribly wrong
A paradigm shift is urgently needed in the way we think about epilepsy in order to significantly impact diagnosis, treatment, prediction and prognosis. Many researchers have considered the hypothesis that epileptogenic and/or ictogenic mechanisms may derive from gradual, but permanent, corruptions of normal neural network behaviour. In fact, such gradual changes could explain the myriad of psychiatric and neurological comorbidities that accompany the epileptic condition, as a grayscale that expands from normality. The active probing of specific circuits could not only highlight dysfunctional behaviour in its early stages but could also evaluate if network activity states are evolving towards seizure onset. Also, proper interference in an epileptogenic/ictogenic network has been shown to block the onset and spread of ictal activity. In this lecture, it will be discussed that all together, such an approach might lead to closed-loop solutions for patients with epilepsy.
FAPEMIG (CBB-APQ-02290-13; CBB-APQ-03261-16; APQ 02485-15), CNPq (307354/2017-2), and CAPES (PROCAD 88881.068460/ 2014-01; BEX 5826/15-2)

L2 – Henry H León-Ariza (Universidad de La Sabana, Colombia)
What is new in the crosstalk between the immune and nervous systems?
In recent years, some researchers have focused on the neuro-immune cross-talk. Primarily, for the regulatory effect of the autonomic nervous system on multiple immune cells as well as the impact of inflammatory mediators on peripheral nerves and directly on the central nervous system. For decades, the sympathetic nervous system has been associated to inflammation. The discovery of adrenoceptors (ARs) on immune cells contributed to understanding this relationship. However, the effect depends on the receptor, it could be: α (inflammatory) or β (anti-inflammatory), besides, the distance from the catecholamine source. In addition, sympathetic nerves release, not only norepinephrine, but other substances as well, as ATP, neuropeptide Y (NPY), or nitric oxide, which influence immune cells. In contrast, the parasympathetic nerves have an anti-inflammatory effect over different cells, via muscarinic and nicotinic acetylcholine receptors, especially through α7-nicotinic acetylcholine receptors (α7nAChRs). Nowadays it is well known that vagal tone is reduced in diseases with an inflammatory component, and for this reason, vagus nerve stimulation is considered an emerging treatment for some inflammatory diseases. Furthermore, the afferent information to the central nervous system uses sensory neurons, which in presence of inflammatory mediators and using a wide group of channels and receptors, leads to enhanced excitability. Besides, an inflammatory process is accompanied by cytokines and other markers that by the hematogenous way reach the hypothalamus and circumventricular organs, areas that affect the autonomic nervous system responses, producing especially an increase of the sympathetic activity and a reduction of the vagal tone. In summary, the immune and nervous system have a close correlation,

which until now has been understood, however, there still remains much to learn from this field. There are doubts about the molecular responses of immune and nervous system cells, especially how the comprehension of this phenomenon can help in the treatment of patients.
Biomedical Physiology Laboratory of the University of La Sabana

L3 - Susan Deuchars (University of Leeds & The Physiological Society (TPS), UK)
Non-invasive neuromodulation of the autonomic nervous system
The maintenance of cardiovascular variables within homeostatic limits is critical for health and is achieved through a major contribution from the autonomic nervous system (ANS). The activity in the sympathetic and parasympathetic branches of the ANS, is finely balanced to maintain appropriate levels of influence over the cardiovascular system when resting, during exercise or in situations such as sleep or stress. As we age or in many pathological situations, there is an impairment in autonomic function, which may contribute to a decline in exercise capacity, quality of life and contribute to disease progression. Indeed, the balance of activity shifts towards sympathetic predominance and this negatively correlates with prognosis in conditions such as heart failure. Therefore, treatments to restore the balance of activity in these two branches of the ANS would be beneficial. There have been many advances in neuromodulatory devices to deliver painless and non-invasive stimulation. We have established the technique of transcutaneous stimulation of the tragus region of the ear to alter autonomic function in humans, shifting the balance towards parasympathetic (vagal) dominance. Importantly, there is a strong correlation between the baseline autonomic activity of each individual and the effectiveness of tragus stimulation. Thus those people with autonomic activity shifted towards sympathetic predominance (commonly observed even in healthy older people), are more likely to see the greatest effects. Interestingly, subjects also reported improvements in measures of quality of life, mood and sleep. Using neuronal tracing and functional studies, we established that the pathways mediating these effects may comprise vagal afferent fibres and other sensory fibres from the great auricular nerve, which innervate the ear and terminate centrally in the brainstem and upper spinal cord. Thus non-invasive neuromodulation may be a simple, inexpensive therapeutic approach to restore autonomic balance in conditions where activity is compromised.
Dunhill Medical Trust, grant number DMT R469/0216.

L4 – Aldo D Mottino (Universidad Nacional de Rosario, Investigador Superior CONICET, Argentina)
Regulation of the intestinal barrier associated with the organic anion-transporter MRP2 by intraluminal nutrients. Role of enteroglucagon GLP-2
The intestinal surface is daily exposed to xenobiotics, including drugs, food additives and contaminants. After absorption of these compounds by the enterocytes, apical ABC transporters play a key role in secreting them back to the intestinal lumen, hence acting as a transcellular barrier. The multidrug resistance-associated protein 2 (MRP2, ABCC2), is an ABC member localized

to the brush border membrane (BBM) of the enterocytes, with maximal expression in proximal jejunum and decreasing towards the distal ileum. MRP2 transports a wide spectrum of amphipathic organic anions preferentially conjugated with glucuronic acid and glutathione. We have explored in rats the influence of dietary components on expression and activity of MRP2, and the potential mediation by glucagon-like peptide 2 (GLP-2), a hormone produced by enteroendocrine L cells in the distal intestine. GLP-2 acts preferentially on proximal intestine by a mechanism only partially understood. We found that sustained increase in food intake, as occurs in lactating mother rats, resulted in increased expression of MRP2 in jejunum and also in distal segments, consistent with improved protection against absorption of xenobiotics all along the small intestine. The mechanism involves long-term, transcriptional regulation, with participation of GLP-2 and intracellular mediation of cAMP, ultimately leading to increased expression of MRP2 mRNA and protein. In more recent studies, we have demonstrated acute, post-translational regulation of MRP2 activity by specific nutrients such as oleic acid and glucose, administered intraluminally to normal rats. The mechanism involves insertion of transporter molecules into BBM, with mediation of GLP-2, and adenosine as extracellular signal acting on the enterocyte. Taken together, our studies demonstrate that food intake (amount and composition) regulates the expression, localization, and activity of intestinal MRP2, and thus its function as intestinal barrier, with GLP-2 being a common, initial mediator. The physiological, toxicological and pharmacological impact of such regulations will be discussed.
The studies were supported by National Agency of Promotion of Science and Technology (ANPCyT), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and Universidad Nacional de Rosario (UNR).

L5 – Hernán Trimarchi (Hospital Británico de Buenos Aires, Argentina)
The podocyte. New concepts
The podocyte is a highly specialized cell located at the outer part of the glomerular basement. It is composed by a cell body and numerous cytoplasmic foot processes known as pedicels, that cover the capillary loops they surround and compose the glomerular filtration barrier together with the basement membrane that connects podocytes with endothelial cells. It is estimated the approximately 500 to 600 podocytes exist in each of the 2 million glomeruli that populate both kidneys. It has been phylogenetically conserved in the evolution of animal species due to its high capability and efficiency to accomplish its main function: To prevent the loss of serum proteins in the urine. Podocytes are uncapable to duplicate and undergo cytokinesis, but can duplicate their nuclear content. Thus, once podocytes are lost, they are lost forever. The critical endowed role to maintain proteins in the circulation is undertaken by a delicate network of specific proteins located at its different compartments. The glycocalyx is mainly involved in intercellular paracrine communication, and its negative charge plays a central role in the permeability of the glomerular filtration barrier. Podocalyxin is located in the glycocalyx itself, and is linked to the cytoplasm by its coupling

with ezrin. The cytoplasmic compartment presents a distinguishing characteristic: The contractile apparatus, composed of actin fibers, myosin, synaptopodin and actinin-IV. The podocyte has the skill to contract or distend depending on the blood flow and intraglomerular pressure. The basal compartment is involved in the attachment to the basement membrane via integrins and uPAR. The stimulation of this tandem can lead to cytoplasmic contraction and podocyte detachment. This podocyte loss in the urine is known as podocyturia, an irreversible process that leads to proteinuria, glomerulosclerosis and chronic kidney disease.

L6 – Angela Myatt (USA)
How to evaluate mobile apps in physiology and health sciences? All that glitters is not gold
Mobile apps have become an integral part of research investigations and healthcare decisions and are in daily use. A million apps are estimated to be available via Google or Apple stores relating to health, fitness, nutrition and wellbeing. Mobile apps designed for instructional purposes are often designed to disseminate information on anatomy and physiology to students in health care professions. In these, the evidence base is a known quantity. What varies is the quality of presentation, ease of use and cost to consumer and producer. In contrast, other mobile apps, such as those dispensing user oriented customized advice and information contain and dispense more subjective information. These mobile apps, often referred to as eHealth, is where I will focus. Many of these apps collect data including physiological parameters from the user, possibly without their knowledge. This may be shared with third parties without the permission of the user. As with any such resource, the information contained within and dispensed by such apps, along with their effects on population and individual health outcomes, needs to be evaluated in an evidence-based manner. This process should in turn be validated and held to a set of standards created by appropriate professional organizations. In this presentation I will describe the history of this category of mobile apps in healthcare and research. I will explore the pivotal issue of regulation and standardization and how this impacts their evaluation, efficacy and validity. I will also discuss the curation of medical apps from the user's perspective. Few rigorously maintained collections exist at present. In conclusion, I will provide participants with a compelling insight into the world of mobile apps. Participants will acquire tools and strategies to employ for evaluation of eHealth apps.

L7 – Daniel Botero Rosas (Universidad de La Sabana, Colombia)
Cerebral blood flow regulation and cerebral hypoperfusion diagnosis in real time using artificial intelligence
Cerebral blood flow (CBF) changes can lead to brain damage. By understanding the mechanisms involved, electroencephalogram and Doppler velocimetry of the middle cerebral artery were obtained. These signals were simultaneously collected from 20 newborns (NB) during states of quiet sleep. Power EEG parameters within frequency bands of interest and de average

velocity of the CBF were estimated at each second. In order to investigate the association in the time (cross correlation-CCF) and frequency domains (magnitude square coherence-MSC) signal processing techniques were developed. During Trace Alternate, CCF between Pθ and Vm resulted in a value of the median of 0.24 in –5 s (Pθ anticipated to Vm) for 84.6% of the NB with $p \leq 0.05$ and the maximum of the MSC median occurred around 0.10 Hz in 92.3% of the NB ($p \leq 0.05$). A distinct behavior was observed for High Voltage Slow. These findings indicate a significant association between the neuronal activity and the CBF during TA. Additionally, in other work, with 88 patients under general anesthesia, values of NIRS, CO2 end-tidal, heart rate, and arterial pressure were obtained and next was classified by physician how hypoperfusion or not hypoperfusion. Next, a neural network multilayer with 50 supervised neurons was implemented and its performance was evaluated. The results obtained were sensitivity (S) of 65%, specificity (SP) of 96%, and C-Statistic (CS) of 82%. These aim to that a machine learning algorithm can be one interesting tool to observe hypoperfusion but is necessary to use other signals how EEG to increase the performance in the diagnosis of hypoperfusion.
Universidad Federal de Rio de Janeiro y Universidad de La Sabana

L8 – Dimitri Mikhailidis (University College London & Editor of Curr Vasc Pharmacol, UK)
Treating hypercholesterolaemia: an update with a focus on evolocumab
Recent lipid guidelines set low density lipoprotein cholesterol (LDL-C) targets (e.g. 55 mg/dl; 1.4 mmol/l) that are difficult to achieve using statins ± ezetimibe. Therefore, the proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9i) will play a key role in reaching these "new" LDL-C goals. In addition, some patients cannot tolerate high dose statins or even any dose of these drugs. It follows that PCSK9i may be an alternative treatment option for these patients. Currently available PCSK9i are the monoclonal antibodies, alirocumab and evolocumab. Their mode of action is to "mop up" PCSK9 in the circulation. This action is of rapid onset. The function of the PCSK9 molecule is to enhance the destruction of LDL receptors (LDLR). It follows that the removal of PCSK9 from the circulation by monoclonal antibodies will increase the number of available LDLRs and thus enhance the removal of LDL-C from the plasma. There is extensive evidence showing that PCSK9i significantly decrease the risk of vascular events (e.g. the FOURIER and ODYSSEY trials). These drugs have a good safety profile. The main adverse event is local irritation at the site of the subcutaneous injection of the PCSK9i (administered once every 2 weeks or every 4 weeks). A PCSK9i can lower LDL-C levels by about 50-60% in patients with coronary artery disease already on statins ± ezetimibe. Their main disadvantage is the cost of treatment. There are also other interesting characteristics of treatment with PCSK9i. These include lowering plasma lipoprotein (a) levels, and potentially beneficial effects on aortic stenosis and venous thromboembolism risk. In the future, there will be a need to also consider the use of inclisiran. This small interfering RNA (siRNA) inhibits the intrahepatic synthesis of PCSK9. The advantage will be that only about 2 injections/year will be needed. Pricing will again be a key issue.

L9 – Linda Samuelson (University of Michigan & American Physiological Society, USA)
Niche regulation of intestinal stem cell function
The intestinal epithelium is continually renewed throughout life by tissue-resident stem cells. Intestinal stem cell (ISC) function is tightly regulated by the microenvironment of signaling factors and cell-to-cell interactions provided by the stem cell niche. My laboratory has used genetic mouse models to investigate niche mechanisms that regulate ISC function during homeostasis and for tissue repair after injury. We have shown that Notch pathway signaling is required for ISC self-renewal and have defined a dynamic cellular remodeling process triggered by pathway inhibition. Epithelial cell responses to Notch disruption include cellular turnover to rebuild the Notch niche cell, as well as activation of facultative stem cells to maintain the epithelium during ISC impairment. The orchestrated response results in rapid return to cellular homeostasis. Our studies demonstrate that the intestine exhibits remarkable cell plasticity to reprogram epithelial cell fate in response to ISC injury, which enables tissue repair and regeneration. Thus, the intestine has great capacity to respond to environmental challenges to maintain tissue function.
Research in the Samuelson laboratory is supported by the National Institutes of Health.

L10 – Juan C Calderón (Universidad de Antioquia, Colombia)
The role of skeletal muscle in the pathophysiology of the metabolic syndrome: muscle mass, fiber types and myokines
The skeletal muscle has been recently associated to the development of chronic metabolic diseases. This implies a change from the classical paradigm that considered the muscle only involved in producing force to a recent view in which it is a metabolically active tissue, that modulates its own metabolism and that of other tissues. We will discuss new results, as well as gaps in knowledge, regarding the possible role of the skeletal muscle in the pathophysiology of the metabolic syndrome: i) the relationship of the regional and global muscle mass with insulin resistance, ii) the impact of the muscle fiber type composition on metabolic health, iii) the role of myokines in the glycemic, lipid and blood pressure control.
Colciencias-Coldeportes (111562638757 from 2014), Colciencias (727 from 2015), IPS-Universitaria-Facultad de Medicina (13041 from 2016), CODI (2565 from 2016) and Banco de la República (4339 from 2019).

SYMPOSIA (S)

S1 – New advances in cardiorespiratory neural control

Coordinator: Rodrigo Iturriaga (Pontificia Universidad Católica de Chile, Chile)

The symposium will discuss recent advances on molecular and cellular physiological mechanisms by which the carotid body chemoreceptors and brainstem nuclei (NTS, RVLM and RTN) plays a key role controlling breathing and cardiovascular function. In the last years, substantial advances in the compression of this process has been obtained using state of the art techniques, including optogenic stimulation, designer receptors exclusively activated by designer drugs (DREADD)-based chemogenomic, and simultaneous recordings of ventilation and blood pressure in free-moving animals. The speakers will discuss a range of topics including the role played by neurons and glial cells in the neural control of cardiorespiratory function in health and disease.

Speakers

Thiago Moreira (University of São Paulo, Brazil)
Adrenergic C1 neurons and the control of cardiorespiratory integration during hypoxia.

Esteban Moya (University of California San Diego, USA)
Microglia activation in the nucleus tractus solitarius with carotid body denervation during hypoxia.

Rodrigo del Río (Pontificia Universidad Católica de Chile, Chile)
Astrocytes from the retrotrapezoid nucleus governs breathing rhythm regulation: implications for disordered breathing in heart failure.

S2 – Muscle as endocrine and paracrine organs

Coordinators: Manuel Estrada (Universidad de Chile, Chile), Gerardo García-Rivas (Tecnológico de Monterrey, Mexico)

Muscle plasticity in response to normal functions and pathological conditions involves circulating hormones and the production and secretions of active factors into muscle cells collectively called myokines and muscle-related hormones. A coordinated link between activation of tissue and several organs are involved excitation-contraction coupling, metabolism, and muscle repair to specific up-to-date endocrine and paracrine muscle actions. Currently, it is known that skeletal myofibers, smooth and cardiac cells produce and release cytokines, myokines, and other active peptides. These molecules play crucial roles in the crosstalk of skeletal and cardiac muscle with other tissues. Together with the synthesis and degradation controlled by muscle metabolites, they represent an essential mechanism to regulate local and whole-body homeostasis. A coordinated link for muscle-related hormone production, secretion action mechanisms, and local and systemic functions has made a current study of endocrine and paracrine muscle actions to be considered a frontier of scientific knowledge and research worldwide. This symposium aims to stimulate the impact of endocrine and paracrine muscle functions.

Speakers

Paola Llanos (Universidad de Chile, Chile)
NLRP3 inflammasome participation in the development of low-grade inflammation during insulin resistance in skeletal muscle

Noemí García (Tecnológico de Monterrey, México)
Physical activity restores the mitochondria organization and function disrupted by obesity in skeletal muscle

Luciana V. Rossoni (Universidade de Sao Paulo, Brazil)
The role of perivascular adipose tissue in the control of vascular tonus

S3 – The exposome and metabolic diseases

Coordinator: Luis Sobrevia (Pontificia Universidad Católica de Chile, Chile)

Exposome refers to environmental contaminants that exert a deleterious effect altering the human health status. The exposome can be external, including air pollution, chemicals in food and water, and diet, and the internal exposome, including age, genetic and metabolic profile. Connections between the function of different organs and the regulation of the functioning of remote organs depend on the type or quality of signalling. Thus, an appropriate immediate microenvironment is required for cell survival, securing a healthy individual. This phenomenon includes the exposome not only from the external environment but of the immediate nearby extracellular environment. Prof David Hill with cover the development and plasticity of the endocrine pancreas responds to both the intrauterine and postnatal exposome in best efforts to predict and respond to alterations in nutritional availability and metabolic requirements. The plastic potential of pancreatic beta-cells appears to be set early in life in response to the exposome but critical windows may exist during the lifespan where the risk of adult metabolic diseases might be reduced through therapeutic interventions. Dr Mari De Angelis and Prof Karl-Werner Schramm will discuss the increasing evidences on how persistent organic pollutants (POP) can interfere with the endocrine system. These POPs referred as "endocrine disrupting chemicals" are widely present in the environment and populations are exposed globally. Perinatal exposure to such chemicals could leads to the onset diseases in later life. It is known, that, maternal thyroid hormones are transported into fetal tissues from 6 weeks of gestation and it seems that during the first trimester, and part of the second, the fetus is entirely dependent on maternal THs supply for its development. The most recent and important clinical studies concerning analytical perspectives, the association and tentative molecular background between the level of thyroid hormones and the exposure to POPs during the perinatal period will be discussed. Prof Heqing Shen will cover the metabolomics for small exogenous and endogenous molecular biomarkers of exposures and their effects (xenobiotics from pollutants and microbes, metabolic adducts and others), adductomics for the measurement of DNA and protein adducts of exposures and effect, computational aided exposure identification to molecule annotation and non-targeting strategies, and biomarker characterization with large-scale data mining and statistical associations among exposures and effects.

Speakers

David Hill (Lawson Research Institute, Canada)
The exposome and pancreatic development and function

Meri De Angelis (Helmholtz Zentrum München-German Research Center for Environmental Health (GmbH), Molecular EXposomics, and Technical University of Munich, Germany)
Perinatal effects of persistent organic pollutants (POP) on thyroid hormone network

Heqing Shen (Xiamen University, China)
Analytical Aspects of Molecular Exposomics

S4 – Preeclampsia more than hypertension in pregnancy

Coordinators: Carlos Escudero (Universidad del Bío-Bío, Chile), Pablo Torres-Vergara (Universidad de Concepción, Chile)

Preeclampsia (PE) is a hypertensive disorder of pregnancy with multisystemic involvement and multifactorial origin that affects between 5-7% of pregnancies in the world, and manifests after 20 weeks of gestation. However, alterations associated to this disease are extended until adulthood in both mother and offspring. Therefore, this disease constitutes a syndrome involving several organs and systems. In this symposium, we joint Latin American speakers belong Chilean (www.grivashealth.cl) and Iberomerican collaborative networks (www.rivatrem.org) who will present evidences from their laboratories. Topics will include

failure in the processes of detoxification; alteration in the coagulation; and placental mitochondrial dysfunction that we hope generates an active interaction with the audience.

Speakers

Carlos Galaviz-Hernández (Instituto Politécnico Nacional, Durango, Mexico)
Association of genetic variants in maternal biotransformation enzymes with preeclampsia
Paola Ayala (Pontificia Universidad Javeriana, Colombia)
Role of thrombomodulin and tissue factor in preeclampsia
Enrique Terán (Universidad San Francisco de Quito, Ecuador)
Mitochondrial activity, ROS and preeclampsia

S5 – Physiology of renal disease

Coordinator: Carlos E Irarrázabal (Universidad de Los Andes, Chile)

In the last two centuries, very important research has been done to understand how the kidneys work and how to treat problems related to their function. All of this research has enabled growth and innovation in kidney care. However, medical evolution has not undergone the advances necessary to decrease the morbidity and mortality associated with kidney disease. In the last two decades. Globally 1.7 million people die from acute kidney disease each year[1]. The global mortality rate from Chronic kidney disease at all ages has increased by 41.5% between 1990 and 2017[2]. This situation establishes the need to continue generating knowledge to prevent and treat these diseases. This symposium will present some of the new advances in Latin American research in this regard.

Speakers

Cristián Amador (Universidad Autónoma de Chile, Chile).
Effect of NGAL on the inflammation produced by unilateral ureteral obstruction
Carlos E Irarrázabal (Universidad de Los Andes, Chile)
During renal ischemia and reperfusion, the absence of TLR4 prevented the markers of Endothelial-to-Mesenchymal markers
Alexis González (Pontificia Universidad Católica de Valparaíso, Chile)
The (pro)renin receptor as a mediator of profibrotic signaling pathways in kidney collecting duct cells

S6 – Endothelial function, metabolism, and signalling

Coordinators: Carlos Escudero (Universidad del Bío-Bío, Chile), Marcelo González (Universidad de Concepción, Chile)

The endothelium forms the inner cellular lining of blood vessels. It is now well established that endothelial cells are highly metabolically active, highly tissue differentiated and play a critical role in many physiological processes, including the control of vasomotor tone, the trafficking of blood cells between blood and underlying tissue, the maintenance of blood fluidity, permeability, angiogenesis, and both innate and adaptive immunity. It is also recognized that the endothelium is involved in most if not all disease states, either as a primary determinant of pathophysiology or as a victim of collateral damage. However, there exists a wide bench-to-bedside gap in endothelial biomedicine. In this symposium, we joint Latin American speakers belong Chilean (www.grivashealth.cl) and Iberomerican collaborative networks (www.rivatrem.org) to discuss about endothelial function, metabolism and signalling. Topics will include O-GlcNacylation, autocrine role in cancer.

Speakers

Fernanda Giachini (Federal University of Mato Grosso, Brazil)
O-GlcNAc impairs endothelial function in uterine arteries from virgin but not pregnant rats: The role of GSK3β

Alejandro S Godoy (Universidad San Sebastián, Chile & Roswell Park Comprehensive Cancer Center, USA)
Angiocrine role of the endothelium in prostate cancer cells
Martha Sosa-Macías (Instituto Politécnico Nacional, Mexico)
Levetiracetam effect on placental carriers in a murine model

S7 – New approaches to identify oncogenic transformation across cancer progression – focus on extracellular vesicles

Coordinator: Carlos Salomón (Universidad de Concepción, Chile & University of Queensland, Australia)

Cancer is defined as abnormal and uncontrolled cell growth and division and is the second leading cause of death globally. Major contributors to cancer-related deaths include lack of accessibility to treatments, delayed diagnosis, and late-stage presentation. Furthermore, metastasis, which is the spread of disease away from the primary site, also contributes to fatalities. Although exact causes underlying oncogenic transformation of normal cells are unknown, it is understood that both genetic and environmental factors contribute to it. Therefore, there is a growing field of research focusing on cancer development, identifying biomarkers, monitoring disease progression, and therapeutic development. The past decade has observed an extraordinary explosion of research in the field of extracellular vesicles (EVs), especially in a specific type of EVs originating from endosomal compartments, called exosomes. In this symposium, we will discuss the potential role of EVs in cancer progression, and the ability of EVs for biomarkers and therapeutics.

Speakers

Andreas Moller (QIMR Berghofer Medical Research Institute, Australia)
Protein content of extracellular vesicles as biomarker in cancer
Andy Tao (Purdue University, USA)
Exosome phosphoproteins for disease diagnosis: Promise and challenges
Shayna Sharma (Early career researcher, University of Queensland, Australia)
Role of exosomes in ovarian cancer

S8 – T-tubules as regulators of heart rhythm and function

Coordinator: Sandra Jones (University of Hull, UK), Matthew Lancaster (University of Leeds, UK)

This session will focus on modulation of the cardiac transverse tubule (T-tubule) system, an extensive structure in cardiac muscle that houses important ion channels and pumps, particularly those involved in the regulation of local calcium concentrations, whilst increasing the overall surface area of the cell and permitting rapid transmission of the action potential into the cell volume. Each speaker will discuss their active research studies of t-tubules, their structural and functional role both in normal cardiac physiology and in specific circumstances such as ageing and disease, with possible therapeutic targets. This will be a stimulating and novel session, providing interest to those involved in cardiac research and general muscle biology.

Speakers

Matthew Lancaster (University of Leeds, UK)
Age-associated loss of t-tubules sets the stage for development of cardiac dysfunction
Katharine Dibb (University of Manchester, UK)
Atrial transverse (t)-tubule and ryanodine receptor remodelling in disease
Cherrie Kong (University of Bristol, UK)
Effects of cardiac caveolin-3 in ageing and heart failure

S9 – Molecular and cellular mechanisms in cardiac diseases

Coordinators: Alicia Mattiazzi (Universidad Nacional de La Plata, Argentina), Paulina Donoso (Universidad de Chile, Chile)

The objective of the symposium is to cover an important area of Cardiovascular Physiology, namely intracellular signals involved in cardiac function and dysfunction. We selected three novel and critical cardiac signalling pathways. One is triggered by Polycystin -1, a mechanosensor that regulates heart contractility and is involved in the regulation of cardiac infarct injury. A second one is triggered by alterations in endoplasmic reticulum function with the consequent activation of several transduction pathways of calcium regulation and dysregulation. Finally, the third one will focus on the link between the immune system and different homeostatic and perturbed conditions in the heart.

Speakers

Zully Pedrozo (Universidad de Chile, Chile).
Polycystin-1 as a new regulator of cardiac infarct injury

Cecilia Mundiña-Weilenmann (Universidad Nacional de La Plata, Argentina)
Endoplasmic reticulum stress: a new player in the pathogenesis of stunned myocardium

Emiliano Medei (Universidad Federal de Rio de Janeiro, Brasil)
Cardioinmunology: The role of IL-1β in atrial fibrillation physiopathology.

S10 – Physiology of extracellular vesicles in cardiovascular disease

Coordinator: Carlos E. Irarrázabal (Universidad de Los Andes, Chile)

Cardiovascular diseases (CVD) consider coronary heart disease, heart failure, stroke, and arterial hypertension. All epidemiological studies by the WHO, AHA / NIH (USA) and MINSAL (Chile) have reported that CVD is the leading cause of death at the local and global level. Worldwide, the WHO reported that in 2012, 17.5 million people died from cardiovascular diseases. The age-adjusted mortality rate goes from 40.4-52.9 to per 100,000 population. Extracellular vesicles are released by cells and contain as nucleic acids, proteins and lipids. Extracellular vesicles rise earlier than troponin in patients with acute coronary syndrome. Exosomes are extracellular vesicles with a size ranging from 30 to 150 nm in diameter and have been suggested as cardioprotectors.

Speakers

Carlos E. Irarrázabal (Universidad de Los Andes, Chile)
Cardiac ischemia induced by stress tests promotes an increase in extracellular vesicles in peripheral blood

Luis Osorio (Universidad de los Andes, Chile)
The nanoparticle tracking analysis technique associated with immunofluorescence allows the quantification of extracellular vesicles with specific charge

Nahuel A García (GECORP, Buenos Aires, Argentina)
Circulating exosomes deliver free fatty acids from the bloodstream to cardiac cells: Possible role of CD36

S11 – TRP channels: health and disease

Coordinators: Enoch Luis Baltazar (Cátedras CONACYT – Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, México), Carlos Fernández-Peña Acuña (St. Jude Children´s Research Hospital, USA)

The transient receptor potential (TRP) channels are a family of ion channels expressed in different tissues of the human body. In mammals, it has been described 28 TRP channels

that are diverse in structure, mechanisms of activation and modulation, and function. The main objective of this symposium is that young/postdoctoral researchers and a Ph.D. student show their main results in the TRP channels field. The talks will cover aspects of TRP channels in neuroscience, including their physiology and pharmacology, and their roles in different processes, like nociception and vascular diseases.

Speakers

Sara Luz Morales Lázaro (Universidad Nacional Autónoma de México, Mexico)
Molecular relationship between TRPV1 channel, the Sigma-1 receptor and progesterone
Rebeca Caires (University of Tennessee, USA)
Omega-3 Fatty Acids Modulate the activity of TRPV4 ion channel through plasma membrane remodeling

S12 – Women in neuroinflammation: A multidisciplinary glimpse from the molecules to the translational medicine

Coordinator: Trinidad A Mariqueo (Universidad de Talca, Chile)

Looking for people's health benefits require a Public Health good design, but also a compromise of the scientist community to look for new tools and approaches in the scientific research. Different disciplines should be connected to develop more approaches in aim to expedite the discovery of new diagnostic tools and treatments. Multi-disciplinary, highly collaborative research groups with different expertise could accomplish that step earlier. Neurophysiology and inflammatory systems share common molecular cues to develop cooperative networks that have been recently involved in central neural system diseases. In this symposium, we propose a new point of view, supported by a strong experimental background in basic science but with an important component on translational science. From the small ionic channel to the individual, several points of view are cooperating to offer 'science with applications' in the neuro-immune field.

Speakers

Fernanda Neutzling-Kaufmann (Université Laval, Canada)
Involvement of CD300f immunoreceptors in major depressive disorder
Carolina A. Oliva (Universidad Andrés Bello, Chile)
Looking for models of neuronal dysfunction to associate with the cognitive decline observed in neurodegenerative diseases
Trinidad A Mariqueo (Universidad de Talca, Chile)
The neuro-immune modulation of inhibitory glycinergic neurotransmission at the central nervous system plays a critical role in the perception of different levels of pain

S13 – Physiological approach to the type 2 diabetes mellitus treatment

Coordinator: Jacobo Villalobos (Hospital Regional de Antofagasta, Chile)

Physiological approach to the type 2 diabetes mellitus treatment", is a great example of integration of the biomedical sciences in regards of the great number of patients all over the world that suffer these diseases and its consequences. Actually, patients can receive a pharmacological treatment elaborated in base of the physiology of the glucose homeostasis, with biochemical and medical evidences of a better patient's prognosis. It is our aim to provide scientific information that support a pharmacological intervention thought since the physiology of hormones, receptors and membranes transporters in different organs that participate in the metabolic control.

Speakers

Daniel Marante (Hospital Regional de Antofagasta, Chile)
Insulin secretion in normal subjects and its abnormalities in Type 2 Diabetes Mellitus and other less common varieties of diabetes.
Jacobo Villalobos (Hospital Regional de Antofagasta, Chile)

Control mechanisms and glycaemia regulation
Cristian Tabilo (Hospital Regional de Antofagasta, Chile)
New drugs in the treatment of DM2 and its effects on the kidney

S14 – Stem cells in regenerative medicine: targeted diseases

Coordinator: Claudio Aguayo T (Universidad de Concepción, Chile)

In recent years a new area of medicine called regenerative medicine has emerged, based mainly on new knowledge about stem cells and their ability to become cells of different tissues. Stem cells are classified as embryonic and somatic or adult. For several years, the hematopoietic stem cell was considered to be the only bone marrow cell with generative capacity. However, several studies have shown that the composition of the bone marrow is complex, with a heterogeneous group of adult stem cells has been identified, among which are: hematopoietic cells, mesenchymal cells, multipotent adult progenitor cells. Several studies have suggested that the potentiality of some types of adult stem cells is greater than expected since they have demonstrated, under certain conditions, the ability to differentiate into cells of different lineages, similar to the potentiality of embryonic cells. This has created new perspectives for the treatment of different diseases, such as type I diabetes mellitus, Parkinson's disease, and myocardial infarction. In this symposium, the basic and clinical concepts of the use of stem cells in the treatment of human diseases will be exposed.

Speakers

Víctor Carriel Araya (Universidad de Granada, Spain)
Biomaterials as stem cells delivery system in peripheral nerve tissue engineering
Paloma Ordóñez-Morán (University of Nottingham Biodiscovery Institute, UK)
Intestinal stem cell niche in both in vivo and novel in vitro models
Patricia A. Luz Crawford (Universidad de los Andes, Chile)
Metabolism regulate the immunotherapeutic potential of mesenchymal stem cells

S15 – The social environment and the physiological state: what changes depending on who is around? Experiences in ruminants

Coordinators: Florencia Beracochea, Julia Giriboni (Universidad de la República, Uruguay)

Animal behaviour influences a wide variety of systems, from neurological to endocrine. In ruminants, social behaviour is inherent to the species, so it is impossible not to consider it when asking new questions and designing experiments. Thus, understanding the physiological bases of behaviour is essential for any researcher in animal science. This symposium proposes to focus on how the social environment determines many of the physiological responses of animals, in particular on reproduction.

Speakers

Rodolfo Ungerfeld (Universidad de la República, Uruguay)
Social environment, endocrine changes and reproductive status
Luis Zarazaga (Universidad de Huelva, Spain)
Physiology of the "male effect" ¿How the male induce the reproductive response on the females?
Antonio Landaeta-Hernández (Universidad de Zulia, Venezuela)
Biostimulation and the restart of reproductive activity in cattle

S16 – Lung cancer: pathophysiological aspects and advances in its treatment. The path from the molecular to the clinical

Coordinators: Ivonne Olmedo, Germán Ebensperger (REECPAL, Universidad de Chile, Chile)

Lung cancer is the leading cause of cancer death in men and second-leading cause of death among women worldwide. Lung cancer is also the cancer with the highest lethality in Latin America and the second-highest lethality in Chile. Factors such as high rates of smoking and poverty as well as a scarcity of knowledge regarding underlying disease mechanisms threaten our ability to control this cancer. Eradicating lung cancer, therefore, represents a series of challenges ranging from basic science to clinical concerns. In our symposium, we will address three topics related to cancer research. Dr López will discuss the role of polyamines in the metabolism of lung cancer tumour cells and the implications of these molecules for the development of cancer. Several cellular processes are involved in the aetiology of this disease, such as epigenetic regulation, cellular proliferation and apoptosis, which ultimately disturb cellular homeostasis. Dr Jara will explain the role of stem cells in lung cancer pathophysiology and the ways in which these cells acquire resistance to drug treatments. Drug resistance greatly hinders the development of therapeutic strategies to combat the primary tumour and subsequent metastases. Finally, Dr Fernández will update us on the progress of clinical research in various therapeutic fields. The panel of researchers will collectively report on our current knowledge regarding the response of tumour cells to the drugs used to fight lung cancer and how physiological or pathophysiological processes are modulated by these experimental approaches.

Speakers

Rodrigo López (Universidad Austral de Chile, Chile)
Polyamines and their role in the metabolism of non-small-cell lung cancer
José Antonio Jara Sandoval (Universidad de Chile, Chile)
Lung tumor stem cells and drug resistance mechanisms
Jaime Gonzalo Fernández (Hospital Clínico Universidad de Chile & Instituto Nacional del Tórax, Chile)
Progress in the treatment of lung cancer: Molecular biology and clinical advances

SYMPOSIUM ABSTRACTS

S1 – New advances in cardiorespiratory neural control

Adrenergic C1 neurons and the control of cardiorespiratory integration during hypoxia.

Milene Malheiros-Lima[1], **Thiago Moreira[1]**, Ana Takakura[2]
(1) University of Sao Paulo, Physiology and Biophysics, Institute of Biomedical Science, 1524 Prof Lineu Prestes Av, Sao Paulo, Brasil.
(2) University of Sao Paulo, Pharmacology, Institute of Biomedical Science, 1524 Prof Lineu Prestes Av, Sao Paulo, Brasil.

Breathing results from the interaction of two distinct oscillators: the pre-Bötzinger Complex (preBötC), which drives inspiration; and the lateral parafacial region (pFRG), which drives active expiration. The pFRG is silent at rest and becomes rhythmically active during the stimulation of peripheral chemoreceptors, which also activates adrenergic C1 cells. We postulated that the C1 cells and the pFRG may constitute functionally distinct but interacting populations for controlling expiratory activity during hypoxia. We found in rats that: a) C1 neurons are activated by hypoxia and project to the pFRG region; b) active expiration elicited by hypoxia was blunted after blockade of ionotropic glutamatergic receptors at the level of the pFRG; and c) selective depletion of C1 neurons eliminated the active expiration elicited by hypoxia. These results suggest that C1 cells may regulate the respiratory cycle, including active expiration, under hypoxic conditions.
FAPESP, CNPq, CAPES-PROEX, Serrapilheira Institute

Microglia activation in the nucleus tractus solitarius with carotid body denervation during hypoxia

Esteban Moya
(University of California San Diego, USA)

Astrocytes from the retrotrapezoid nucleus governs breathing rhythm regulation: implications for disordered breathing in heart failure.

Rodrigo del Río
(Pontificia Universidad Católica de Chile, Chile)

S2 – Muscle as endocrine and paracrine organs

NLRP3 inflammasome participation in the development of low-grade inflammation during insulin resistance in skeletal muscle

Paola Llanos[1,2]
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(2) Universidad de Chile, Center for Exercise, Metabolism and Cancer Studies (CEMC), Facultad de Medicina, Independencia 1027, Independencia, Santiago, Chile

Introduction: Areas of active investigation focus on the molecular bases of chronic low-grade inflammation and potential pathogenic roles during insulin resistance. The activation of the NLRP3 inflammasome has been proposed as one of the pathways to promote chronic low-grade inflammation in various tissues including adipocytes and liver. In skeletal muscle, however, a direct demonstration of a link between insulin resistance and the NLRP3 inflammasome is unknown. Aim: The aim of this investigation was to characterize the level of protein expression of NLRP3 complex and its product, IL-1β, in skeletal muscle from insulin resistance mice. Methods: Male C57BL/6J mice were fed with normal diet (NCD) or high fat diet (HFD) for 8 weeks. The proteins NLRP3, ASC, caspase 1, gasdermin-D (GSDMD) and IL-1b were analyzed using Western blot in homogenized of Flexor digitorium brevis (FDB) and Soleus (Sol) muscle from NCD and HFD-fed mice. NLRP3 inflammasome components were localized in isolated fiber by indirect immunofluorescence. Caspase-1 activity was measured using a fluorometric assay. All experiments were performed with n=3-6. Values were expressed as the mean ± SEM. Statistical significance was calculated using the Mann-Whitney test, and a value of $P \leq 0.05$ was considered statistically significant. Results: An increase in protein levels of NLRP3 and ASC, activation of caspase 1 and mature IL-1b were found in muscle homogenates of FDB and Sol from HFD compared to NCD-fed mice. The pore-forming protein GSDMD (as the conduit for IL-1b secretion to cytosol) also was increased in HFD mice. Interestingly, we located NLRP3 complex within isolated fiber, suggesting that is not only expressed in skeletal muscle fibers, but also it could have an active role in this tissue. Conclusion: Insulin resistance is related to an increase in levels of NLRP3 inflammasome components in the adult skeletal muscle of obese animals. ACKNOWLEDGMENT: FONDECYT 1190406
Support by: FONDECYT 1190406.

Physical activity restores the mitochondrial organization and function disrupted by obesity in skeletal muscle
Noemí Garcia[1]
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Introduction: Obesity alters the structure and mitochondrial function. Mitochondrial structure impaired has been attributed to unbalanced mitochondrial dynamics (mtDym) [1] that on a micrography of skeletal muscle can be observed as small mitochondria [2]. It's known that physical activity (PA) increases the oxidative capacity and also have evidence that regulates the mtDym [3], [4]; nevertheless, there is still little evidence in obesity conditions. In non-muscle cells, the mitochondrial networks are observed easily in cells live, however in skeletal muscle mitochondrial networks cannot be visualized clearly; nevertheless, as these are associated with T-tubules [5] it is feasible to do analyzed it. Objective: To evaluate the effect of PA and exercise moderated on the mitochondrial organization in gastrocnemius muscle from obese rats. Material and Methods: Male Zucker fa/fa rats were divided into sedentary and subjected-to-PA (one session of swimming) or exercise moderated (4 weeks of swimming), with the approval of the ethics committee, # register 2019-007. Mitochondrial organization and proteins related to mtDym were evaluated in gastrocnemius muscle fibers by confocal microscopy and western blot. Unpaired student's test or one-way ANOVA was performed to compare experimental groups, $p<0.05$ was considered significantly different. Results: The PA increased the transversal organization level of the mitochondria analyzed in isolated fibers, which correlated with the increase in Mtf2 ($p<0.05$) and a decrease in Drp1 ($p<0.05$) and OMA1 ($p<0.05$), without changes in AMPK levels. On the other hand, after exercise moderated restored the transversal organization of subsarcolemmal mitochondria. The analysis of all mitochondrial density did not show significant changes. Besides, Drp1 ($p<0.05$) and AMPK-P (Thr172) ($p<0.05$) were found increased. Conclusion: Restoration of mitochondrial organization in skeletal muscle from obese rats is dependent on time and intensity of PA as well as the AMPK activation that modulated the activity of proteins related to mtDym.
Acknowledgment: Funded by the group of Medicina Cardiovascular y Metabolómica, Tecnológico de Monterrey.

The role of perivascular adipose tissue in the control of vascular tonus
Luciana V. Rossoni
(Universidade de Sao Paulo, Brazil)

S3 – The exposome and metabolic diseases

The exposome and pancreatic development and function
David Hill[1]
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The development and plasticity of the endocrine pancreas responds to both the intrauterine and postnatal eposome in best efforts to predict and respond to alterations in nutritional availability and metabolic requirements. Both under- and over-nutrition in utero can lead to a mismatch in pancreatic hormonal demands and hormone-secreting capacity postnatally, This can be further exacerbated by metabolic stress postnatally resulting from obesity or pregnancy, resulting in an increased risk of gestational diabetes, type 2 diabetes, and even type 1 diabetes. The plastic potential of pancreatic beta-cells appears to be set early in life in response to the eposome but critical windows may exist during the lifespan where the risk of adult metabolic diseases might be reduced through therapeutic interventions. Since the ability of functional beta cells to undergo proliferation is limited in adulthood the potential maximum beta cell mass appears to be set early in life, with a finite capacity for endocrine cell neogenesis from resident progenitor cells within the pancreatic duct lining and scattered throughout the exocrine tissue. This progenitor pool can be induced to proliferate and differentiate into new beta cells in response to enhanced metabolic demand. The response can be mediated through locally produced and acting growth factors and cytokines including insulin-like growth factors, fibroblast growth factors, the apelinergic system and glucagon-like polypeptide 1 (GLP-1). Both the size of the endocrine progenitor cell pool and the expression and actions of the trophic molecules can be impaired both in utero and in childhood to limit the adaptability of the beta cell mass under metabolic stress in adult life.
The Lawson Foundation Canadian Institutes of Health Research

Perinatal effects of Persistent Organic Pollutants (POP) on Thyroid Hormone Network.
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(2) Technical University of Munich, Department für Biowissenschaftliche Grundlagen, Weihenstephaner Steig 23, 85350 Freising, Germany
Thyroid hormones (TH) are known to play a critical role in regulating many metabolic processes including the correct fetal growth and development. Over the past two decades, increasing evidences have shown that certain persistent organic

pollutants (POP) can interfere with the endocrine system. These POPs referred as "endocrine disrupting chemicals" are widely present in the environment and populations are exposed globally. However, the association of low-exposure POPs with thyroid hormones (THs) during the perinatal period still remains unclear. To better elucidate the possible relationship between some POPs and THs, we have investigated the association among: (i) placental levels of various POPs and thyroid hormones (THs) and (ii) breast milk level of the same POPs and thyroid hormones. The concentrations of polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins/furans (PCDD/Fs), organotin chemicals (OTCs), organochlorine pesticides (OCPs), T4, T3, and rT3 were measured. Several POPs were significantly associated with THs in placenta: (1) T4 was inversely associated with BDEs 99, 100, and 2378-TeCDD, and positively associated with 1234678-HpCDF; (2) T3 was positively associated with 2378-TeCDF and 12378-PeCDF; and (3) rT3 was positively associated with PCB 81, 12378PeCDF, and 234678-HxCDF, and inversely associated with tributyltin and methoxychlor. In the breast milk: (1) T4 was inversely associated with BDE-99, -154, and -196; (2) T3 was inversely associated with BDE-47, -99, -100, -197, -203, -207, and OCDD; and (3) rT3 was inversely associated with BDE-47, -99, -183, and -203. These studies provide new insight into the complexity of thyroid-disrupting properties of POPs, although more research is needed to elucidate the biological consequences of such exposure.

Analytical Aspects of Molecular Exposomics

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The ethic available biological samples such as blood and urine from the investigated population have convened all possible information of global metabolome including exposures and the related responses. Therefore, metabolomics could encompass the totality of the exposure and the linkages to diseases. In addition to endogenous metabolites, recent advances in analytical techniques have led to the detection of various xenobiotics that are usually undetected using traditional targeted methods. The high-throughput metabolomics is an untargeted analysis encompass a wider range of chemicals, in which the unbiased measurement has combined with the systematic character of the matrix of blood or urine. The advanced ultra-high-resolution (>60,000) mass spectrometers (UHRMS) with adapted algorithms (for processing complex mass spectral data such as the over 100,000 chemical signals in blood) and human metabolism database will be the important techniques for the measurement. In addition, protein adductomics and DNA adductomics are the important complementary to metabolomics, which are the major information traps of the biological active chemicals. The traditional targeted analyses measure the target chemical pollutants, their metabolites, or reaction products in the biological species with high confidence. Measurement of the listed chemicals by the integrated high-throughput technologies such as general unknown screening (GUS) has also developed, which majorly used in the clinical and forensic fields. Up-to-date, the UHRMS can either match the unknown sample features to compounds by suspect screening analysis (SSA) or elucidate structures of unknowns that may not be contained in a database by non-targeted analysis (NTA), these developing approaches can be practically combined with the various exposomics models, such as the top-down and bottom-up paradigms, the environment-wide association study (EWAS), the meet-in-metabolite analysis (MIMA), the sample pooling strategy and so on. In summary, to address the final purposes for exposomics will improve the identification of causes of diseases and benefit the health preventive strategies.

S4 – Preeclampsia more than hypertension in pregnancy

Association of genetic variants in maternal biotransformation enzymes with preeclampsia.

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Introduction: N-acetyltransferase (NAT2) and epoxide hydrolase (EPHX) are biotransformation enzymes involved in placental oxidative stress. Despite placenta has maternal and paternal genetic contributions, the last has been poorly studied. Objective: to determine the association of biparental genetic nucleotide variants (NV) of NAT2*5/*6 and EPHXrs2234922 A>G with preeclampsia (PE). Methodology: this cases-control study was approved by the ethics and research committees of the Health Ministry in Durango, México and included 55 women affected with PE and their partners and 45 women with healthy pregnancy and their partners. The population was sub-divided into three groups: women-only, men-only and combined (women/men). The analysis included genotyping of NAT2*5 (C>T) /*6 (G>A) and EPHXrs2234922 A>G nucleotide variants through real time PCR. Comparisons of categorical variables were performed using chi-square and/or Fisher's exact tests. The intergroup comparisons were analyzed with the Mann-Whitney U test. The association between the

polymorphism and the disease was evaluated through multivariate regression analysis. Results. NAT2*6 "A" allele was significantly associated with preeclampsia (OR: 2.10, IC 95%:1.09-4.04, p=0.023) under a recessive inheritance model, in the group of partners but not in the mothers. EPHX rs2234922 "G" allele showed a protective effect for PE (OR 0.23; 95%IC 0.08-0.69, p=0.0055) in the mother´s group under a dominant inheritance model. Conclusions. NAT2*6 "A" allele was significantly associated with PE in the group of partners but not in the mothers, meanwhile a protective effect was observed for "G" allele of EPHX rs2234922. These results highlight the importance of evaluating the maternal / paternal binomial in PE.
This work was supported by grants from Secretaría de Investigación y Posgrado (SIP) of Instituto Politécnico Nacional (SIP 20201479 and SIP20201482).

Role of thrombomodulin and tissue factor in preeclampsia
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(Pontificia Universidad Javeriana, Colombia)

Mitochondrial activity, ROS and preeclampsia
Enrique Terán
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S5 – Physiology of renal disease

Effect of NGAL on the Inflammation Produced by Unilateral Ureteral Obstruction
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Background: The renal inflammation has been proposed as a crucial mechanism of renal disease, where the macrophages recruitment depends of different chemokines/cytokines increase. Studies have demonstrated that neutrophil gelatinase-associated lipocalin, NGAL, it is overexpressed during early stages of renal lesion. However, whether NGAL is relevant for macrophages recruitment, and if this is related to the increase of pro-inflammatory mediators at renal level, remains unknown. Objective: To determine whether NGAL promotes the pro-inflammatory status during the unilateral ureteral obstruction (UUO).Methods. Male C57BL/6 Wild-type (WT) and NGAL-KO mice were undergoing to UUO and to Sham surgery during 3, 7 and 14-days (n=8). All the procedures were approved by the Ethical Committee of Universidad Autónoma de Chile. Results are represented by mean±SEM, and data were analyzed by ANOVA-test. Results: In WT mice, the UUO induced tubular dilation starting at 3-days (P<0.001 vs. Sham), and an induction of plasma urea (76.36 ± 5.37mg/dL. P<0.01 vs. Sham). UUO increased NGAL levels in mononuclear cells (PBMC), plasma and urine (24.1µg/L in Sham vs. 103.8µg/L and 134.5µg/L in UUO, at 3 and 7-days, respectively). This was in accordance with the renal induction of NGAL (mRNA and protein, P<0.001 vs. Sham), and with the increase of mRNA for the following pro-inflammatory mediators: TGF-β1, CCL5 and MCP-1, with a peak at 7-days. The genetic abalation of NGAL prevented tubular dilation and the MCP-1 rise induced by UUO in kidney and PBMC (P<0.001 vs. WT). This was accompanied with a lower macrophage infiltration and by a slower fibrosis progression in kidney of NGAL-KO mice underwent to UUO. Conclusion: The renal overexpression of MCP-1, the macrophage recruitment, and the velocity of fibrosis induced by UUO is dependent of NGAL. These results suggest that NGAL may be crucial for the pro-inflammatory phenotype driving to fibrosis in renal disease. Acknowledgments. Fondecyt #1201251

During renal ischemia and reperfusion, the absence of TLR4 prevented the markers of Endothelial-to-Mesenchymal markers
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Introduction: renal ischemia and reperfusion (I/R) cause acute kidney damage and Toll-like membrane receptors (TLRs). The TLR4 receptor plays a critical role in inflammation and reparation during ischemia. The endothelial-to-mesenchymal (EndMT) is observed in process of I/R Therefore, to understand the molecular mechanisms associated with the role of TLR4 during kidney I/R damage will help to know in deep the function of this receptor. Objective: to determine the role of TLR4 during the renal I/R in the upregulation of markers of eEndMT transition using a Wild type (Wt) and Knockout (KO) animals for TLR4. Methodology: male C57BL/6 Wild type (Wt) and Knockout (KO) mice for TLR4 from 2-3 months of age were subjected to a model of renal I/R. The ischemia and reperfusion were caused by 30 min. of ischemia and 48 hours of reperfusion. Sham controls were used. Vimentin, Fascin-1, and HSP47 (rRT-PCR and Western blot). The kidney damage was evaluated by NGAL (Western blot) and histology (Hematoxylin and Eosin and Periodic Acid Schiff stained). Results: the results with histological analysis showed that both groups (Wt and KO) showed tubular damage (loss of tubular structure and decreased brush border) induced by I/R. NGAL was increased by I/R in the cortex and medulla of Wt and KO animals. The Vimentin and Fascin-1 expression was upregulated by I/R in the cortex and medulla of WT animals. The TLR-KO prevented the Vimentin and

Fascin-1 upregulation by I/R. The Hsp47 did not show changes in gene expression by I/R and the TLR4-KO did not change this pattern. Conclusions: the absence of TLR4 during the I/R renal did not significantly prevent kidney injury (NGAL) and tubular damage, but prevented the upregulation of EndMT markers, suggesting that this process is dependent of TLR4.
FAI, Corfo PI-2676

The (pro)renin receptor as a mediator of profibrotic signaling pathways in kidney collecting duct cells
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The binding of prorenin to the (pro)renin receptor (PRR) triggers the activation of MAPK/ ERK1/2 pathway, induction of cyclooxygenase-2 (COX-2), NOX-4-dependent production of reactive oxygen species (ROS), the induction of transforming growth factor β (TGF-β), connecting tissue growth factor (CTGF) and plasminogen activator inhibitor (PAI-I) in collecting duct (CD) cells. PRR activation increases profibrotic factors through COX-2-mediated PGE2 activation of E prostanoid receptor 4 (EP4), upregulation of NOX-4/ROS production, and activation of Smad pathway in mouse CD cells. We have shown in several studies that recombinant prorenin increased ROS production and protein levels of CTGF, PAI-I, and TGF-β in M-1 CD cell line. Inhibition of MAPK, NOX-4, and COX-2 prevented this effect. Inhibition of MEK, COX-2, and EP4 also prevented the upregulation of NOX-4. COX-2 inhibition or EP4 antagonism significantly prevented phosphorylation of Smad 2/3 Wichmann is activated by TGFF-β. Mice infused chronically with recombinant prorenin showed an induction in the expression of CTGF, PAI-I, TGF-β, fibronectin, and collagen I in isolated collecting ducts as well as the expression of alpha smooth muscle actin (α-SMA) in renal tissues. COX-2 inhibition prevented this induction. These results indicate that the induction of TGF-β, CTGF, PAI-I, and ROS occurs through PRR-dependent activation of MAPK and NOX-4; however, this mechanism depends on COX-2-derived PGE2 production and the activation of EP4 and Smad pathway.
FONDECYT 1191006

S6 – Endothelial function, metabolism, and signalling

O-GlcNAc impairs endothelial function in uterine arteries from virgin but not pregnant rats: The role of GSK3β
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Angiocrine role of the endothelium in prostate cancer cells
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Levetiracetam effect on placental carriers in a murine model
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Introduction: Levetiracetam (LEV) is an antiepileptic drug indicated in pregnant women. The impact of LEV on placental carriers is poorly understood. Objective. To assess the effect of LEV exposure on the mRNA expression of placental carriers for hormones, nutrients, and drugs in a murine model, and correlate their expression with the drug's serum concentration. Methodology: Pregnant Balb-c mice were distributed randomly in two control and two experimental groups of 10 mice each (total n=40). The experimental groups received 100 mg/kg of LEV every 24 hours since weaning. Mice in the four groups were crossed and sacrificed on gestational days (GD) 13 and 18; on which serum LEV measurements were performed by HPLC-UV. The placental expression of carriers Slc7a11, Lat1, Slco4a1, Folr1, Slc19a1 and Slc38a4 was evaluated through qRT-PCR. Differences in gene expression were determined with the Mann-Whitney U test, using the $\bar{x}$ RQ value of each group. The correlation between serum LEV concentrations and placental gene expression was evaluated using Pearson's correlation coefficient. Results: At GD 13, no differences on gene expression was observed between those exposed to LEV and the control group. Conversely, at GD 18 the expression of Slc7a11, Lat1, Slco4a1 and Slc38a4 was higher in the group treated with LEV regarding the control group ($p<0.05$), while the expression of Slc19a1 was lower ($p=0.003$). A negative correlation was observed between the expression of Folr1, Slco4a1, Slc7a11 and Slc19a1 carriers and the serum concentration of LEV ($r= -0.64$ to -0.67, $p<0.01$), in contrast, the correlation with Slc38a4 was positive ($r=0.52$, $p=0.032$). Conclusions: The present study suggested that LEV alters the expression of placental carriers implicated in the exchange of nutrients and hormones, at GD 18. Further studies are warranted to determinate the potential effect of LEV both in the model studied and in the human.
Acknowledgments: this research was funded by grants from the Secretaría de Investigación y Posgrado (SIP) of Instituto Politécnico Nacional (SIP 20201479 and SIP 20201482).

S7 – New approaches to identify oncogenic transformation across cancer progression – focus on extracellular vesicles

Protein content of extracellular vesicles as biomarker in cancer

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Despite significant therapeutic advances, cancer remains a leading cause of death worldwide. A significant clinical problem is the generally late discovery of a cancer and the uncertainty of choosing the most effective therapy for the individual patient. Several novel biomarkers are proposed, ranging from genetic and genomic evaluations of the cancer or the cancer material in circulation to assessing nucleic acids, proteins or lipids. Accurate cancer biomarkers, in particular non-invasive liquid biomarkers based on blood samples or other body fluids, will allow clinicians to identify cancer patients early, triage them to the most appropriate intervention and follow the response of the cancer in real time over the course of the therapy. In this presentation, I will share data on a novel biomarker for Non-Small-Cell Lung Cancer (NSCLC). We have developed a blood-based multi-protein signature capable of accurately prognosticating clinical outcome in NSCLC patient cohorts. This signature is contained in small circulating nano-vesicles termed exosomes. Overall, this work describes a novel prognostic biomarker signature to identify early stage NSCLC patients at risk of developing metastatic NSCLC, thereby enabling implementation of personalized adjuvant treatment decisions.

Exosome phosphoproteins for disease diagnosis: Promise and challenges

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The state of protein modification can be a key determinant of cellular physiology such as early stage cancer. Here we present a strategy to isolate and identify phosphoproteins in extracellular vesicles (EVs) from human biofluids such as plasma, urine and saliva as potential markers to differentiate disease from healthy states. We developed a robust workflow for biofluidic EV cargo analysis, and identified thousands of phosphoproteins in EVs isolated from small volumes of biofluid samples. Using label-free quantitative proteomics, we identified a large number of phosphoproteins in plasma EVs that are significantly higher in disease patients as compared to healthy controls. Several novel biomarkers were validated in individual patients using Paralleled Reaction Monitoring for targeted quantitation. This study demonstrates that the development of phosphoproteins in plasma EV as disease biomarkers is highly feasible and may transform cancer screening and monitoring.

Role of exosomes in ovarian cancer

Shayna Sharma

(Early career researcher, University of Queensland, Australia)

S8 – T-tubules as regulators of heart rhythm and function

Age-associated loss of t-tubules sets the stage for development of cardiac dysfunction

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With increasing age the incidence of cardiac arrhythmias and poor cardiac function also increases. It is against this background of the aged heart that most cardiac pathology presents. At the gross anatomical scale hypertrophy is commonly seen and a degree of fibrosis. At the microanatomical scale individual myocyte hypertrophy is evident with ventricular cells from mice at 24 months of age being on average 41% wider and 20% longer than cells from animals at 6 months of age. Looking at higher resolution, despite an age associated increase in cell capacitance accompanying the myocyte hypertrophy transverse tubule (t-tubules) structures are progressively lost with density reducing by 18% across the same age range. This age-associated loss of t-tubules associates with a significant reduction in the intracellular calcium transient amplitude and a slowing of the rise and decay time of the transient. Looking across the myocyte the rise of the transient becomes significantly more heterogeneous in advanced age reflecting poor coordination in the recruitment of sarcoplasmic reticulum (SR) calcium release. Examination of t-tubule structure reveals marked 'empty' areas in mycoytes from animals at extreme ages (> 27 months). Such loss of t-tubules might be anticipated to lead to significant disruption of communication between the SR and surface membrane and significant increases in the numbers of spontaneous calcium release events (SPARKS) are observed in myocytes from aged animals compared with those from young animals with the individual amplitude of each significantly reduced. Overall the data suggests loss of t-tubule structure in old age contributes to poor function and is likely a contributor to the increased risk of arrhythmias seen in old age. Thanks to the British Heart Foundation who supported this work.

Atrial transverse (t)-tubule and ryanodine receptor remodelling in disease.

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Transverse (t)-tubules are regular deep invaginations in cardiac cell membranes around which the Ca release machinery is concentrated. The rise in intracellular Ca, which initiates contraction, occurs as Ca entering the cell on the L-type Ca channel triggers the release of a large amount of Ca from the intracellular Ca store, the sarcoplasmic reticulum (SR). Efficient Ca release requires L-type Ca channels, on the cell surface membrane or t-tubule, to be closely associated with the clusters of SR Ca release channels known as ryanodine receptors (RyRs). The space between the L-type Ca channels and RyR clusters is known as the dyad. As well as being present in ventricular myocytes, t-tubules occur in the atria of large mammals, including humans. Using a sheep model, we have shown atrial t-tubules, and therefore central dyads, are almost entirely lost in heart failure (HF) contributing to the decrease in Ca transient amplitude. Super resolution microscopy (STORM) reveals that in addition to t-tubule remodelling, in HF, the localization, size and spacing of atrial RyR clusters are altered. We propose that both t-tubule loss and RyR remodelling contribute to the decrease in Ca transient amplitude found in HF. With this in mind, we have sought to determine if it is possible to restore t-tubules and RyR organisation in HF. Confocal microscopy shows t-tubules are restored, although disordered, and RyR localization is improved following recovery from HF. Our data suggests t-tubule restoration and improved RyR localization can restore the systolic Ca transient in atrial myocytes.

British Heart Foundation

Effects of cardiac caveolin-3 in ageing and heart failure

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Introduction: The aged heart is more prone to failure, and many molecular changes are common to both conditions. Caveolin-3 (Cav-3) is a membrane-associated protein that has been suggested to be important for t-tubule integrity and L-type Ca current (ICa) regulation, while changes to t-tubule structure and function have been strongly implicated in ageing and the development of heart failure (HF). Goals: To investigate the possible involvement of Cav-3 in ageing and HF.
Methodology: T-tubule structure, ICa and Ca release were compared in ventricular myocytes obtained from aged mice (3 vs 24 mo), and from mice that had undergone transverse aortic constriction (TAC) to produce cardiac insufficiency. Cav-3 expression was altered using transgenic knock out (KO) or over-expression (OE). T-tubular ICa,L density was determined using a 'detubulation' method. Statistical analyses were performed using t tests, ANOVAs or their non-parametric equivalents. All animal procedures were performed in accordance with UK legislation. Results & Conclusions: Ageing was associated with a small reduction in Cav-3 expression, t-tubule density and whole-cell ICa density, where the latter occurred almost exclusively within the t-tubules. TAC and Cav-3 KO produced similar, but more dramatic, effects. Cav-3 OE had no detectable effects on cell size or t-tubule organization, but prevented the age-dependent redistribution of ICa. In TAC, Cav-3 OE reduced cellular hypertrophy, prevented the ICa redistribution, and limited the decrease in the rate of rise of the whole-cell Ca transient. These data suggest that Cav-3 is not directly involved in t-tubule maintenance, but appears to play a role in ICa redistribution in disease and ageing. Given the importance of Cav-3 in signalsome construction, it is possible that these changes in ICa may be a consequence of changes in (for e.g.) β-adrenergic or connected signaling complexes.

S9 – Molecular and cellular mechanisms in cardiac diseases

Polycystin-1 as a new regulator of cardiac infarct injury

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Polycystin-1 (PC1) is a mechanosensor crucial to maintain the cardiac function. Mice harboring cardiomyocyte-selective conditional silencing of PC1 (PC1 knockout, PC1-KO) have a decreased ejection fraction and fractional shortening. Moreover, PC1-KO do not develop cardiac hypertrophy or fibrosis after being subjected to transverse aortic constriction. On the other hand, cardiac fibroblast primary cilia and PC1 also are required for cardiac fibrosis after apical resection, ischemia/reperfusion (I/R), or myocardial infarction injury, suggesting that this protein may contribute to cardiac remodelingCardiac I/R damage is the major cause of cell death in the heart. During and after I/R, signals such as mechanical stress regulates cardiomyocytes to induce survival or cell death through different pathways, however the role of PC1 during

cardiac ischemia/reperfusion was not studied. In vivo and ex vivo I/R and simulated in vitro I/R (sI/R) were induced in PC1-KO mice and PC1-knockdown (siPC1) neonatal rat ventricular myocytes (NRVM), respectively. Infarcts were larger in PC1-KO mice subjected to in vivo and ex vivo I/R, and necrosis rates were higher in siPC1-NRVM than control after sI/R. Moreover, PC1 activated the pro-survival AKT protein during sI/R and induced PC1-AKT-pathway-dependent CTGF expression, a profibrotic factor during I/R. Together, these data suggest that PC1 mitigates cardiac damage during I/R, likely through AKT activation, and regulates CTGF expression in cardiomyocytes via AKT. PC1, therefore, may emerge as a new key regulator of I/R injury-induced cardiac remodeling.
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Endoplasmic reticulum stress: a new player in the pathogenesis of stunned myocardium
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The endoplasmic reticulum (ER) is a calcium storage compartment primarily involved in folding and sorting of newly synthesized secretory and membrane proteins. Different cellular perturbations affect ER function leading to accumulation of unfolded/misfolded proteins and frequently ER calcium leakage. To restore homeostasis, ER triggers various signaling pathways called the unfolded protein response (UPR). If the UPR fails, a terminal UPR program ensures the cell commitment to self-destruction. ER stress is emerging as key contributor to many human diseases, including ischemic heart disease. Reperfusion of ischemic myocardium, although necessary to salvage tissue from eventual death, aggravates the extent of ischemic injury, damage collectively known as ischemia/reperfusion (I/R) injury. Clinical manifestations of I/R injury ranges from reversible contractile dysfunction and arrhythmias (stunned heart, brief ischemia), to cell death (prolonged ischemia). Increasing evidence shows that severe I/R challenges the ER protein folding capacity leading to pro-apoptotic UPR pathways activation and cell death. However, little was known about the ER stress response during a mild I/R insult which causes minimal or no cell death. In a perfused rat heart stunned model we found that both, cytoprotective and pro-apoptotic UPR pathways were activated. Prevention of ER stress by chemical chaperones, resulted beneficial for the post-ischemic mechanical recovery, despite no attenuation of I/R-induced oxidative stress. Moreover, inhibiting ER stress mediated-calcium release was also protective. Blockage of the translocon, one of the main ER calcium leak channel, precluded ER stress and improved the post-ischemic contractile recovery. Overall, our results suggest that ER stress via stimulating calcium leakage from the ER, contributes to the altered calcium homeostasis, which underlies the mechanical dysfunction of the stunned myocardium. Modulation of translocon permeability appears as a yet not explored therapeutic approach to face dysfunctional consequences of the I/R injury.
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Cardioinmunology: The role of IL-1β in atrial fibrillation physiopathology.
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Introduction: Atrial fibrillation (AF) is the most frequent cardiac arrhythmia. Several cases of AF patients present an increased level of interleukin 1-β (IL-1β). Aims: This work aims to test the hypothesis that IL-1β is involved in the physiopathology of AF. Methods: We compared C57BL/6 mice subcutaneously daily-treated with IL-1β or with vehicle (Control) for 15 days. Atrial action potential (AP) and Ca2+ transients were recorded by Local Field Fluorescence Microscopy and Subcellular Optical Mapping, at an intact heart level. Results: IL-1β treated mice showed shorter atrial AP duration (APD) when compared to control hearts. Ca2+ transients had faster kinetics in IL-1 β treated mice. Rise time (RT): 12.9 ± 2.9 vs. 10.7 ± 2.8 Time to peak (TP): 20.4 ± 5.6 ms vs. 16.0 ±0.9 ms and fall time (FT): 51.8 ± 2.4 ms vs 48.1 ± 2.0 ms. After perfusion with ryanodine and thapsigargin, Ca2+ transient amplitude (CTA) had a higher reduction in IL-1β treated hearts. ΔCTA: 85.5 ± 5.7 % vs 95.5 ± 3.0 %. Additionally, the IL-1β treated group presented a higher number of arrhythmic triggered events after an S1-S2 simulation protocol (Control = 1/10 vs. IL-1β = 7/13 mice). Furthermore, Subcellular Optical Mapping of the membrane potential shows a heterogeneous spatial distribution of the APD in the appendix of the left atrium. Conclusion: In conclusion, the results presented suggest that IL-1β produces changes in APD and Ca2+ signaling. Moreover, the decrease in the amplitude of Ca2+ transients can induce a shortening of the APD due to decrease activation of the Na+- Ca2+ exchanger in its forward mode. Finally, the shortening of the APD will induce a faster recovery of Na+channels from voltage-dependent inactivation promoting a scenario where AF is more likely to occur.
FAPERJ, CAPES and CNPq

S10 – Physiology of extracellular vesicles in cardiovascular disease

Cardiac ischemia Induced by Stress Tests promotes an increase in extracellular vesicles in peripheral blood.

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Introduction. Cardiovascular diseases (CVD) are the main causes of death globally. Coronary artery disease (CAD) is the most common type of heart disease. The CAD happens when the arteries that supply blood to the heart muscle become hardened and narrowed (cardiac ischemia). We previously established that extracellular vesicles (VEs) are upregulated in peripheral blood samples earlier (2hrs) than cardiac Troponin I (cTnI) in patients with Acute Coronary Syndrome (ACS) from the emergency room. The Stress Test is used in clinic activity to the diagnosis of cardiac ischemia but has limited sensitivity (around 70%). Objective: To evaluate if the quantification of VEs in the blood samples is associated with cardiac ischemia in patients under Stress test to suggest a complementary method to diagnosis this heart condition. Methodology: Peripheral Blood samples were obtained from 46 patients subjected to cardiac ischemia provocation (Stress Test) in the Cardiology Unit of the Clínica Dávila. Blood samples were obtained in a time course of 30 min (0, 15, and 30 min), once the Stress test was concluded. The determination of the concentration of different sizes of VEs in plasma derivate from peripheral blood samples. The concentration of EVs of different sizes was carried out by NTA (Nanoparticle Tracking Analysis) using the Nanosight equipment. Results. The EVs were present in all the blood samples analyzed. The patients with cardiac ischemia had increased levels of EVs in blood samples (3.5 times; 0.74 versus 2.56 x 1012 / mL plasma; p <0.0002) than negative patients at 0, 15, and 30 minutes after finished the Stress test. The small vesicles had the best correlation with cardiac ischemia. Conclusions. The quantification of EVs by NTA in peripheral blood samples is a novel tool to help in the diagnostics of cardiac ischemia. Limitations and advantages are discussed. Corfo PI-2676

The nanoparticle tracking analysis technique associated with immunofluorescence allows the quantification of extracellular vesicles with specific cargo.

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Introduction: The diagnosis of acute myocardial infarction (AMI) is made based on the increase in plasma levels of cardiac troponin (cTn), which occurs 3-4 hours after the start of AMI when there is already tissue damage due to necrosis. It is of clinical interest to have a marker of cardiac ischemia, prior to cardiomyocyte necrosis. Previous results from our laboratory showed an increase in the concentration of extracellular nanovesicles (VEs) in peripheral blood of patients with acute coronary syndrome (ACS), during the first 2 hours in the emergency unit of Clínica Dávila, time in which the cTn is not yet detectable, suggesting that the release of NVEC is an event prior to the release of the cTn (cell necrosis). Interestingly, the elevation of VEs was an earlier event than the elevation of cTn (myocardial infarction biomarker) in patients with ACS, these data suggest that the increase in the concentration of VEs in peripheral blood is a pre-infarction event to the heart. On the other hand, it is described that cardiomyocyte derived VEs contain exosomes markers as CD81. To determine the levels of CD81 and a potassium channel present in cardiac cells, associated with VEs in peripheral blood of patients at Clínica Dávila subjected to cardiac ischemia caused by cardiac stress test. Methodology: Peripheral blood was processed and used for nanoparticle tracking analysis (NTA), coupled with the detection by indirect immunofluorescence of CD81 and the potassium channel, preliminary results (n=2). This study was approved by the ethical-scientific committee of Clínica Dávila. Results. CD81 and the potassium channel positive VEs increases in 7,2% and 5,7% respectively in patients with cardiac ischemia. Conclusion. The proteins evaluated by NTA in this project are increased in ischemic patients and could be proposed as biological markers of cardiac ischemia. Acknowledgment. Funded by project CORFO PI-2676 and Clínica Dávila.
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Circulating exosomes deliver free fatty acids from the bloodstream to cardiac cells: Possible role of CD36

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S11 – TRP channels: health and disease

Molecular relationship between TRPV1 channel, the Sigma-1 receptor and progesterone

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Introduction: The TRPV1 ion channel has been widely associated with the generation of painful and itchy responses. One way to regulate the effects of TRPV1 is to control the amount of these ion channels in the plasma membrane. Some studies have reported that the interaction of TRPV1 with intracellular proteins regulates TRPV1 levels located on the cell surface and the pain produced through its activation. Objective: Explore the association of TRPV1 with a chaperone protein, such as the Sigma-1 receptor (Sig-1R). Methods: This work was perform using TRPV1-expressing HEK293 cells and primary cultures of DRG neurons. The methods used here were: whole-cell patch-clamp recordings, total and cell-surface protein isolation, WB and coimmunoprecipitation assays, Confocal Microscopy Analysis, FRET Measurements, Sig-1R–knockdown Experiments. The behavioral pain studies were carried out using C57BL/6 mice, which were handled following the protocol approved by the Institutional Animal Care and Use Committee of the Instituto de Fisiología Celular of the National Autonomous University of México (UNAM; IACUC protocol TRF17-14). Statistical comparisons were made using the ANOVA or Student's t-test, and $p \leq 0.05$ was considered statistically significant. Group data are reported as the mean ± SEM. Results: We demonstrate a physical interaction between TRPV1 and Sig-1R. We found that progesterone negatively regulates the expression of TRPV1 through the disruption of the Sig-1R / TRPV1 complex. Finally, we showed that TRPV1-dependent pain decreases through the antagonism of Sig-1R by progesterone. Conclusion: The results shown here constitute an important way to regulate TRPV1-mediated pain by progesterone and its protein association with Sig-1R.
This work was supported by DGAPA-PAPIIT Grant IA202717 and Beca para Mujeres en la Ciencia L'Oréal-UNESCO-CONACYT-AMC.

Omega-3 Fatty Acids Modulate the activity of TRPV4 ion channel through plasma membrane remodeling
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Omega (ω-3) polyunsaturated fatty acids (PUFAs) consumption is an important part of the human diet because their multiple health benefits. ω-3 PUFAs reduces vasoconstrictors effect, preventing vascular dysfunction. Eicosapentaenoic acid (EPA), an ω-3 PUFA found in sea food products, has antithrombotic and anti-inflammatory effects on the cardiovascular system. The health benefits of ω-3 PUFAs have been demonstrated, however, the molecular mechanism by which these fatty acids exert their effect remains unknown. PUFAs and their metabolites are known to modulate membrane receptors, as well as ion channels. Arachidonic acid (AA), an ω-6 PUFA, and its metabolites (EETs), generated in the cascade after deformation of the membrane by mechanical stress, activates the transient receptor potential vanilloid 4 (TRPV4). TRPV4 is a polymodal ion channel, essential for the control of vascular tone and blood flow. For a better understanding of the molecular mechanisms by which TRPV4 modulates vascular reactivity, it is essential to identify the metabolic pathways that modulate its function. In this research we study whether ω-3 PUFAs modulate the function of TRPV4, thus regulating vascular activity. Using Caenorhabditis elegans (C elegans), an animal model that allows precise manipulation of the lipid environment of the membrane. We performed a screening for different PUFAs in these worms, finding that EPA and its derivative, epoxyeicosatetraenoic acid (17, 18-EEQ), are required for the function of TRPV4. We demonstrated that TRPV4 activity is negatively regulated in those membranes that have a low level of PUFAs. Using electrophysiology, calcium imaging and mass spectrometry, we demonstrate that EPA increases the amount of TRPV4 channels available to be activated. Differential calorimetry (DSC) and atomic force microscopy (AFM) demonstrate that ω-3 PUFAs increase the membrane fluidity and decrease bending stiffness. Our proposed model suggests an increased TRPV4 function by metabolism of ω-3 PUFAs and the remodeling of the membrane.

S12 – Women in neuroinflammation: A multidisciplinary glimpse from the molecules to the translational medicine

Involvement of CD300f immunoreceptors in major depressive disorder
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Introduction: Major depressive disorder (MDD) is recurrent and debilitating psychiatric disorder that affects many people worldwide, with a higher prevalence in women when compared to men. Studies have shown that MDD is associated to immune system dysregulations driving an increased inflammatory profile. The CD300f immunoreceptors are inhibitory

receptors expressed by microglial cells and can produce anti-inflammatory responses. The absence or pharmacological blockage of CD300f receptors potentiates inflammatory and autoimmune diseases. Aim: To evaluate the involvement of CD300f immunoreceptors in MDD in mice and humans. Methods: We evaluated 5 and 18-month-old CD300f knockout mice (CD300f-/-) in several behavioral paradigms related depression-like behavior. Hippocampal microglia cells were isolated by FACS from control and LPS (2 mg/kg) treated mice and the RNAseq were performed. Hippocampal neurotransmitter levels were analysed by HPLC. The polymorphism (rs2034310 C/T) on CD300f immunoreceptor were evaluated in a cross-sectional population-based study including 1.111 subjects. Data are presented as mean ± SEM and $p<0.05$ was considered significant. The study was approved by the Ethical committee. Results: Behavioral analysis showed that 5 and 18 months old female CD300f-/- mice presented depressive and anhedonic behavior. The 5-month-old CD300f-/- females presented altered IL-6, IL1RN and IL-10 expression indicating a moderate neuroinflammatory status accompanied by decreased hippocampal noradrenaline levels. Acute treatment with bupropion (10 mg/kg, i.p.) improved while LPS injection (2 mg/kg i.p.) exacerbated anhedonic-like behavior in CD300f-/- females. RNAseq data demonstrated that the absence of CD300f induces downregulation of metabolic pathways such as glycolysis, gluconeogenesis, mitochondrial fatty acid β-oxidation, lipid synthesis and Krebs cycle. In humans, the T allele from CD300f polymorphism was associated with protection against MDD in women.Conclusion:CD300f immunoreceptors present a role in MDD with a sex-specific approach and may be useful for an accurate diagnosis and discovery of new therapeutic targets.

Looking for models of neuronal dysfunction to associate with the cognitive decline observed in neurodegenerative diseases

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Some of the most socially relevant diseases, such as neurodegenerative diseases are correlated with the degeneration of specific circuits in the brain, causing emotional disturbances and cognitive impairments that progressively worsen until death. We use in vitro and in vivo models that can generate different types of oscillatory activity circuit-specific, to investigate at the level of cells, circuit and neuronal networks, the progression of major neurological diseases, and to predict if some brain circuits are more susceptible than others to suffer neuronal dysfunctions. We use the entorhinal cortex-hippocampal circuit to study Alzheimer's disease (AD), the prefrontal cortex circuit for schizophrenia, and the striatal-cortical circuit for Huntington and Parkinson's diseases. To study synaptic transmission and integration, we combine electrophysiological recordings of cortical neurons with the microscopic imaging of dendritic spines, to visualize the activity of cellular ensembles along different postnatal ages, depending on the onset of the particular disease's model. Moreover, we study whether the pattern of protein expression, such as pro-inflammatory molecules in a specific region, affects specific functional properties of that circuit. The subtypes of amyloid-β (Aβ) peptides are metabolic products released in an activity-dependent form. We are developing an in vitro tool to study different forms of amyloid-β (Aβ) present in patients, to correlate whether the aggregation state of Aβ generates a pro-inflammatory state that triggers the disease in the patient.

The neuro-immune modulation of inhibitory glycinergic neurotransmission at the central nervous system plays a critical role in the perception of different levels of pain

Trinidad A Mariqueo

(Universidad de Talca, Chile)

S13 – Physiological approach to the type 2 diabetes mellitus treatment

Insulin secretion in normal subjects and its abnormalities in Type 2 Diabetes Mellitus and other less common varieties of diabetes.

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Introduction: Insulin secretion by beta cells is a complex and tightly regulated process; its integrity is essential for a normal glucose homeostasis. As opposed to T1DM, caused by a destruction of beta cells, Type 2 Diabetes Mellitus (T2DM), Neonatal Diabetes (ND) Maturity Onset Diabetes of the Young (MODY) are rather due to dysfunction of beta cells. Objective: To review the mechanism of insulin secretion in the normal subject and to describe the current view about its derangements in diabetes mellitus. Methodology: Review of the fundamental literature regarding insulin secretion mechanisms in order to pave the way for a physiological understanding of the treatment of diabetes mellitus. Results: In T1DM the absolute insulin deficiency is due to an autoimmune destruction of beta cells. Therefore, the treatment of T1DM follows this concept. Regarding T2DM, a number of facts are well established; although there is some ethnic heterogeneity, generally speaking it can be said that insulin resistance is a major pathophysiological defect. However, insulin resistance alone does not lead to T2DM unless a defect of insulin secretion adds on, and the mechanism is not entirely understood. Secondary defects are also described, such as glucotoxicity. ND is a consequence of mutations that cause dysfunction of the SUR-Kir 6.x complex on the membrane of the beta cell. Mutations of the Glucokinase gene lead to MODY 2 sub-type; this condition is usually

benign and can be managed with a minimal intervention. Conclusion: The cause of the abnormalities of insulin secretion in DM are diverse. Its recognition has allowed to adjust treatments in a more informed way, targeting the main pathophysiological defects. Self-financed

Control mechanisms and glucemia regulación

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Introduction: healthy subject´s serum glucose concentration is submitted to a rigorous control; it is usually between 80-90 mg/dl in the fasting state, liver is an important glycemic regulator. Insulin and glucagon functions as an antagonic feedback system, essential to keep glycemic normal range. Chatecolamines secreted by adrenal glands improve hepatic glucose release, protecting from acute hypoglycemia; growth hormone and cortisol, are released as a response to prolonged hypoglycemic. Hyperglycemic, produces osmotic diuresis and systemic dehydration. Diabetes mellitus enhances risk for cardiovascular disease, ictus, chronic renal failures, and blindness. Objective: To analyze physiological mechanisms of glycemic control and regulation, to approach T2DMMethology: review of the literature related to the glycemic control and regulation mechanisms based on the new drugs for type 2 diabetes mellitus treatment. Results: There is a very fine glycemic regulation that starts from the cephalic fase of the digestion with ghreline secretion by gastrointestinal cells, which inhibits insulin and activates glucagon synthesis. These actions are antagonized by GLP-1, which enables insulin secretion and acts in CNS too, reducing apetite and enhancing saciety. GLP-1 can be inactivated by DPP4. Glucose is absorbed in the enterocyte by the SGLT1 transporter, with a sodium cotransport; the same mechanism exists in the proximal contoured tubule of the nephron, allowing the reabsorption of the filtered glucose, maintaining the glycemia and avoiding glucosuria. Glucose can enter the myocyte by the GLUT4 transporter, activated by insulin, however, PPARγ can generate modifications in the transporter conformation and prevent the input of glucose to the myocyte. Conclusion: physiologically, glycemia is maintained in a normal range, by a multisystemic self-regulated hormonal system enhanced by transporters and intracellular pathways, allowing osmotic and energetic equilibrium of the organism as a whole. elf-financed

New drugs in the treatment of DM2 and its effects on the kidney

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Introduction: CKD increases cardiovascular mortality 2-3 times and diabetic nephropathy explains 40% of hemodialysis. Objective: To identify the effect of iSGLT-2-aGLP-1 in diabetic nephropathy. Methodology: Literature review Results: Hyperglycemia generates glomerular hyperfiltration through hemodynamic alterations that appear in the early stages of diabetes. At the level of the proximal tubule, in the T2D patient, an increase in glucose reabsorption via the SGLT2 cotransporter is observed, which ultimately leads to vasodilation of the afferent arteriole, with increased intraglomerular pressure and hyperfiltration. This phenomenon has also been related to an increase in the expression of pro-inflammatory and profibrotic factors that lead to kidney damage. ISGLT-2 act at the proximal tubule level, inhibiting glucose and sodium reabsorption by approximately 70%, restoring sodium concentration at the level of the macula densa, which, by means of an increase in adenosine, produces vasoconstriction of the afferent arteriole. ISGLT-2 and aGLP-1 not only have a hypoglycemic effect, but have also been shown to reduce cardiovascular and renal morbidity and mortality. In addition, cardiovascular safety clinical studies have shown to decrease the incidence or progression of nephropathy, the progression to macroalbuminuria, double baseline creatinine, and start dialysis. Regarding aGLP-1, there is still no clarity of the mechanism of action; it probably has a natriuretic effect due to inhibition of the NHE3 transporter in the proximal tubule, associated with a decrease in HbA1c%, blood pressure and weight. Clinical studies also show a decrease in the deterioration of kidney function. There is no evidence about the combined use and improvement of cardio-renal outcomes. Conclusions: The iSGLT-2 and aGLP-1 are currently the first line in the treatment of patients with DM2 with high cardiovascular risk, for the reduction of cardiovascular events, progression of kidney disease, and mortality. self-financied

S14 – Stem cells in regenerative medicine: targeted diseases

Biomaterials as stem cells delivery system in peripheral nerve tissue engineering

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Introduction: Peripheral nerves (PNs) are crucial organs that functionally connect the central nervous system with distal target organs. PNs structure and function are often affected by traumatic injuries and their repair remains a major challenge for surgeons. In this context, engineered substitutes developed by the tissue engineering emerged as a promising alternative for these patients. Objective: to investigate the use of nanostructured fibrin/agarose hydrogels nerve substitutes (NFABNS) as adipose-derived mesenchymal stem cells (ADMSCs) delivery system in peripheral nerve repair. Methods: Here, 20 adult male Wistar rats were used for the isolation of ADMSCs and to create 10-mm left sciatic nerve defects under general

anesthesia. Defects were repaired by using autografts (Auto control group), cellular NFABNS (Nano group), and Neuragen® conduits filled with cellular NFABNS, five healthy animals were used as control (n=5). After 4 and 12 weeks, animals were subjected to functional analyses (pinch test of sensory recovery, toe-spread test, and electromyography (EMG)), whereas muscle morphometry and nerve histology were conducted at 14 weeks. Results: Functional analyses after 4 weeks confirmed the motor, sensory and electrophysiological compromise in all groups, but also revealed some signs of reinnervation in the Auto group. After 12 weeks, functional improvements were observed with a decrease of the denervation and a comparable increase of the reinnervation between Auto and Nano groups. At 14 weeks, muscle morphometry showed, important atrophy, especially in Coll-Nano (56.8% weight) followed by Nano groups (54.4% weight) and Auto groups (32.67% weight) ($p<0.05$). Histology confirmed the presence of an active PN regeneration process with the positive reaction for S-100, GAP-43, and myelin in all experimental groups. Discussion: This in vivo study suggests that the NFABNS could be a promising alternative in peripheral nerve repair obtaining a closely comparable recovery profile than the use of the autograft technique.
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Intestinal Stem Cell Niche in Both in Vivo and Novel in Vitro Models
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Aberrant activation of some pathways can create hierarchically organized tumour tissues where a subpopulation of self-renewing cancer stem cells sustain the long-term clonal maintenance of the neoplasm. These cells have proven to be resistant to conventional therapy and to be responsible for tumour relapse. My objective is to target these cells based on their stem-like properties and thereby identify efficient approaches for cancer therapies to improve patient survival. To this end, I use mouse models, clinical association analyses, high-throughput approaches, next-generation sequencing, single-cell technologies, patient-derived material, and 3D organoids.

Metabolism regulate the immunotherapeutic potential of mesenchymal stem cells
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Mesenchymal Stem/Stromal Cells (MSC) are promising therapeutics tools for inflammatory diseases due to their potent immunoregulatory capacities. Their suppressive activity mainly depends on inflammatory cues that have been recently associated with changes in MSC bioenergetic status towards a glycolytic metabolism. However, the molecular mechanisms behind this metabolic reprogramming and its impact on MSC therapeutic properties have not been investigated. To this aim, we reprogramed the metabolism of murine and human MSC using pro-inflammatory cytokines, an inhibitor of ATP synthase (oligomycin), or 2-deoxy-D-glucose (2DG). We found that the oligomycinmediated pro-glycolytic switch of MSC significantly enhanced their immunosuppressive properties. Conversely, glycolysis inhibition using 2DG significantly reduced MSC immunoregulatory effects. Moreover, in vivo, MSC glycolytic reprogramming significantly increased their therapeutic benefit in the delayed-type hypersensitivity and graft versus host disease mouse models. Finally, we demonstrated that the MSC glycolytic switch effect partly depends on the activation of AMPK-HIF-1alpha signaling pathway. Altogether, our findings show that AMPK- HIF-1alpha dependent glycolytic reprogramming of MSC using an ATP synthase inhibitor contributes to their immunosuppressive and therapeutic functions, and suggest that pro-glycolytic drugs might be used to improve MSC-based therapy.

S15 – The social environment and the physiological state: what changes depending on who is around? Experiences in ruminants

Social environment, endocrine changes and reproductive status.
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The sudden introduction of males to anestrous females stimulate the secretion of LH, the ovarian follicular development, and ovulation, even during the non-breeding season, in sheep, goats and cattle (the “male effect”). This type of stimulus can also modify other physiological functions, as in cattle and goats can advance the luteolytic process. On the other hand, the sudden introduction of estrous females induce the secretion of LH and testosterone in rams and bucks (the “female effect”). If this is prolonged over time, semen quality can also be enhanced. However, the presence of other male can inhibit the reproductive activity of rams, bulls and bucks, mainly due to hierarchical bonds. In this sense, the mere presence of a dominant male can inhibit the sexual display of a subordinate one, or trigger modifications in their courtship strategy. If the males did not know each other, joining them is an important stressor that triggers increases in cortisol, body temperature,

and affect semen quality for several days. The induction of sexual behavior in anestrous ewes or does can also stimulate ovulations in anestrous females that remain in close contact. Moreover, joining females from different breeds that differ in their seasonal pattern can shorten the anestrous period in the more season ones. However, dominant does can inhibit the reproductive responses of subordinate ones. In general, the signals involved and the consequences on the reproductive physiology have been used to improve the productive results in these species. However, little is known on how these effects can interfere in research studies.

Physiology of the "male effect" ¿How the male induce the reproductive response on the females?
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The "male effect" is a practice used usually in extensive or semiextensive small ruminants farm systems during the seasonal anoestrous. This practice consists in a sudden exposure of anovulatory females to males resulting in a stimulation of the reproductive activity. Nevertheless, the isolation between males and females it has been demonstrated that is not necessary when sexually active males are used. This introduction induces a rapid GnRH increase pulse secretion that stimulates the LH pulse frequency, followed by a preovulatory LH surge and ovulation. The induction of the LH surge is nearly always due to an increase in estradiol secretion and modification on the sensitivity of the hypothalamus-pituitary-gonadal axis (HPG) to the negative feedback of estradiol. The mechanisms and ways involved in the regulation of this network are very complex. Visual stimuli undoubtedly play an important role, but it seems that plays only a supplementary role in the "male effect". Vocalizations emitted by sexually active males during courting behavior alone do not seem to be able to fully stimulate the HPG axis. In both cases the full contact between males and females induces a higher reproductive response. The olfactory signals, involving pheromones or some molecules that stimulates the main olfactory system are clearly involved. In this way, the 4-ethyloctanal is responsible of the activation of the GnRH pulse generator in female goats. The sexual behavior of the male is another key factor on the response to the "male effect". All those signals are integrated and acts on different brain regions including olfactory bulb, amygdala and noradrenergic neurons in the locus coeruleus and A1 nucleus. They influence hypothalamic kisspeptin neurons which in turn then activate GnRH neurons. The precise neural networks linking the various cues associated with the "male effect" to the GnRH neurons are not completely established.
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Bioestimulación y reinicio de la actividad reproductiva
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El efecto biostimulatorio ocurre a consecuencia de una señal feromonal y otras señales no olfativas. En vacunos, la vía de emisión de esa señal parece ser orina. Estudios sugieren que las señales pudieran asociarse a moléculas solubles del complejo de histocompatibilidad mayor (CHM) que pueden transportar moléculas odoríferas alelo-específicas desde la sangre a la orina. Estas moléculas transportadoras son degradas en orina, donde liberan moléculas volátiles que portan olores específicos del CHM que serán detectados por el sistema olfatorio para desencadenar respuestas neuroendocrinas en las hembras que las perciben. La captación y conducción de la señal es por vía oro-nasal (flehmen, conducto nasopalatino y órgano vomeronasal). La señal es disuelta en la mucosidad del órgano vomeronasal y reconocida por neuronas y receptores específicos V1r de la zona apical y V2r en capa basal del neuroepitelio. El sistema olfatorio principal es el principal responsable de captar la señal feromonal del macho, pero otras señales no olfativas parecen estar involucradas. Seguidamente, neuronas secretoras de GnRH representan la conexión entre el sistema olfatorio, hipotálamo, amígdala y cerebro medio quienes reaccionan a las señales bioestimulatorias y la cascada de transducción alcanza al sistema límbico e hipocampo. Finalmente, el estatus endocrino es alterado mediante la secreción de LH, la que pudiera representar un estímulo para luteinizar pequeños folículos y generar el primer de progesterona necesario para restituir la actividad ovárica. Parece posible un booster energético causado por cortisol y NEFA. La amígdala también contiene receptores para esteroides gonadales y adrenales que pueden estar involucrados con procesos de conducta y feedback con la adenohipófisis para liberar ACTH y LH. Las señales feromonales del macho pueden mejorar la proporción de células secretoras de GnRH que co-expresan FOS luego de la estimulación.

S16 – Lung cancer: pathophysiological aspects and advances in its treatment. The path from the molecular to the clinical

Polyamines and their role in the metabolism of non-small-cell lung cancer
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Introduction: Polyamines (putrescine, spermine and spermidine) are small cations essential for tumor proliferation. In non-small cell lung cancer (NSCLC), they have been proposed as early markers of tumorigenesis. Enzymes involved in polyamine synthesis, such as ODC and AMD1, are among the first proteins increased in tumorigenesis. On the other hand, SSAT, the enzyme that catabolizes polyamines can be increased with antitumor effects. So, polyamine metabolism has become an attractive chemotherapy target. Aim: The aim of our work is to study the metabolic effects of polyamine metabolism-targeted drugs on NSCLC cells, and to explore the synergistic profile over cell viability when these drugs are used in combination. Methodology: Our approaches include comprehensive synergistic profiling, by using the COMBENEFIT software, where the viability data (obtained by MTT reduction) is uploaded. Also, we analyze the metabolic effects of drugs by GC/MS metabolomics and the polyamine-related set of proteins is analyzed by western blotting. Results: We have explored inhibitors of the main enzymes related with polyamine metabolism: DFMO (an ODC inhibitor), SAM486A and everolimus (which inhibit and decrease protein levels of AMD1, respectively) and non-steroidal anti-inflammatory drugs (NSAIDs), which can increase SSAT levels. Also, we have assayed MDL72527 and methoctramine, inhibitors of the recovery polyamine process from their acetylated state. Among the multiple combinations assayed, we have gotten interesting results with the NSAID indomethacin. Indomethacin increase the levels of SSAT, affecting the arginine metabolism, including polyamine-related pathways and shows synergistic effects with methoctramine, suggesting the acetylation and further exporting of polyamines as main mechanism of synergy. Interestingly, these effects seem to be associated with the mutation profile of NSCLC cell lines. Conclusion: Our data shows that a multiple-targeted therapy targeted to polyamine metabolism is a suitable strategy to improve NSCLC chemotherapy. However, further studies about the genetic background influence over drug response is needed. FONDECYT grants #1160807 and #1201378

Lung tumor stem cells and drug resistance mechanisms

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Lung cancer is the most common cancer in men and the leading cause of cancer death among both men and women worldwide. Actually, patients undergoing cancer treatments, the survival rates remain extremely poor (5-years), due to the development of resistance and relapse. Cancer Stem Cells (CSC) subpopulation is favored by hypoxic environment. These cells are responsible for tumor initiation, progression, cell death resistance, chemo- and radiotherapy resistance, and tumor recurrence. In this sense, CSC mitochondria has been emerging as a cancer therapeutic target because play a crucial role for the regulation of cell death and bioenergy regulation. Our objectives are to characterize the presence of CSC in hypoxic cultures of lung tumor cells and to evaluate Benzoic acid lipophilic cations, as cytotoxic molecules, in combination with doxycycline, in lung tumor cells cultures in hypoxic conditions. Through flow cytometry we are evaluating stemness-related markers. Cell viability was determined in both normoxic and hypoxic conditions using MTT reduction assay. Combination assays were performed using diverse benzoic acid derivatives with doxycycline through Combenefit software and further LOEWE analysis to determine the cytotoxic effect of the combinations. Mitochondrial functions were evaluated trough mitochondrial transmembrane potential ($\Delta\Psi m$) and intracellular ATP content. One-way or two-way ANOVA was used as statistical analysis. Our results showed that exist a subpopulation of CSC in hypoxic cultures of lung cells and TPP+C10 have cytotoxic effect on hypoxic lung tumor cell lines and some combinations (1.31 µM TPP+C10 with 0,5 to 5 µM range of Doxycycline) showed synergic effects ($p<0.05$). Additionally, all benzoic acid derivatives induced a decrease in both intracellular ATP (15%; $p<0.05$) and $\Delta\Psi m$ ($p<0.05$).Hypoxic cultures are more resistant to cytotoxic molecules probably because CSC subpopulations present. TPP+C10 showed synergic effects in some combination with doxycycline in hypoxic conditions. These results will be confirmed in in vivo models.
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Progress in the treatment of lung cancer: Molecular biology and clinical advances

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Introduction: Lung cancer is the leading cause of death by cancer worldwide, and from 2018 including Chile. It is a seldom tractable disease, except for early stages in which a combination of surgery and/or Chemo/radiotherapy achieves survival of 60-40% at 5 years. The advances stages (more than 70% at diagnoses) have been traditionally treated with palliative measures or Chemotherapy. However, in the last 10 years, an explosion of new therapies has arrived, targeting different pathways of cancer cell signalling. Aims: We will discuss new insights in lung cancer cell biology, the main drivers of tumoral progression and how these mechanisms are targeted. Finally, we will talk about how the new drugs and techniques yields progresses in survival and quality of life of patients. Results: In the last 10 years a lot of bench basic knowledge has been transferred to clinical improvements at the bedside. Most notably, targeting of EGFR pathway and PD1/PDL1 signalling has more than doubled survival of stage IV patients, once candidates to palliative treatment only. Discussion: The advances in the treatment of lung cancer has changed the paradigms of this lethal disease. New questions are arriving, at the molecular, clinical, public health and financial levels.

YOUNG RESEARCHERS SYMPOSIA (YRS)

YRS1 – Aquaporins in physiological and pathological conditions

Coordinator: Raúl A. Marinelli (Instituto de Fisiología Experimental (IFISE-CONICET), Universidad Nacional de Rosario, Rosario, Argentina)

The aquaporins (AQPs) are a family of intrinsic membrane channel proteins that facilitate the osmotically-driven movement of water molecules. Some AQPs also display permeability to certain small uncharged molecules. AQPs assemble into tetrameric functional units, essential to life, being expressed in all kingdoms. In humans, there are 13 AQPs, at least one of which is found in every organ system. The structural biology of the AQP family is well-established and many functions for AQPs have been reported in health and disease. The targeted modulation of AQPs therefore presents an opportunity to develop novel treatments for diverse conditions or reliable diagnostic and prognostic biomarkers. This symposium aims to highlight the relevance of AQPs in the context of some pathological conditions such as neuromyelitis optica, cholestasis and preeclampsia.

Speakers

Natalia Szpilbarg (Assistant Researcher, CONICET, Laboratorio de Biología de la Reproducción, IFIBIO UBA-CONICET, Universidad de Buenos Aires, Argentina)
Possible role of AQP3 in the etiology of preeclampsia

Vanina Netti (Assistant Researcher, CONICET. Laboratorio de Biomembranas, IFIBIO UBA-CONICET, Universidad de Buenos Aires, Argentina)
Role of aquaporin-4 as an osmosensor in retinal müller cells: implications in the physiopathology of neuromyelitis optica spectrum disorder

Julieta Marrone (Assistant Researcher, CONICET. Instituto de Fisiología Experimental, IFISE-CONICET, Universidad Nacional de Rosario, Argentina)
Hepatic gene transfer of Aquaporins for cholestasis

YRS2 – Biology of exercise in metabolic disorders

Coordinator: Sergio Martínez Huenchullán (Universidad Austral de Chile, Chile)

Obesity is a global health problem, where its high prevalence worldwide, and particularly in Latin America, is associated with the development of metabolic complications ranging from insulin resistance and type 2 diabetes, to cardiovascular disease, and non-alcoholic fatty liver disease. Therefore, strategies that aim to counter these obesity-derived complications have been in increasing develop in the last decades. From those, physical exercise is one of the most effective lifestyle modifications used to manage obesity and its comorbidities. However, even when its health benefits are well-known, the physiological mechanisms behind these processes are far from being fully understood. Organ-cross talk, redox signalling, and insulin-signalling are some of the processes being currently investigated. The motivation behind the scientific exploration of this knowledge gap resides in develop advances towards the individualization of exercise modalities as therapy for metabolic disorders. This symposium aims to highlight some of the recent advances in the understanding of the physiological mechanisms and effects of exercise in the context of metabolic dysfunction, particularly focused on obesity and insulin resistance, along with some of the future challenges in the field.

Speakers

Carlos Henríquez-Olguín (Postdoc, University of Copenhagen, Denmark)
Intracellular hydrogen peroxide as signal for molecular responses to exercise.

Jonas Roland Knudsen (Postdoc, École Polytechnique Fédérale de Lausanne, Switzerland)
Novel insights into GLUT4 in insulin-sensitized and –resistant skeletal muscle

Sergio Martínez-Huenchullán (Lecturer-Researcher, Austral University of Chile, Chile)
Influence of exercise intensity on metabolic adaptations in an obesity context.

YRS3 – Protein and membrane interaction, always a good interaction? Osmoionic imbalances and signalling mechanisms in pathological contexts

Coordinator: Pablo J. Schwarzbaum (Principal Researcher CONICET - Instituto de Química y Fisicoquímica Biológicas "PROF. ALEJANDRO C. PALADINI", Argentina)

Exposure to toxins from different organisms can trigger a variety of responses in different cell types. In human erythrocytes, both exposure to the peptide Mastoparan-7 and exposure to the toxin alpha-hemolysin (HlyA, secreted by uropathogenic strains of E. coli (UPEC), activate signalling mechanisms mediated by extracellular ATP and induce an osmotic imbalance in these cells. On the other hand, it has been shown that HlyA in an important virulence factor in the pathogenesis of urinary tract infections in pregnant women, where UPEC strains are responsible of the 80% of the infections. Exposure to HlyA of human chorioamniotic membranes induce the remodelling of the extracellular matrix, leading to a premature birth or abortion.

It has been suggested that the damage induce by toxin-membrane interaction might be mediated by the alteration of water homeostasis, among other mechanisms, due to the interaction of the toxins with one or more aquaporin present in the cell membrane. This interaction would trigger lytic mechanisms in different tissues during the course of a bacterial infection or a toxin-induce cellular injury. Since the discovery of aquaporin 1 (AQP1, the main water transport channel in human erythrocytes) and each member of the AQP family, it has been studying the role of these transmembrane channels in the sensing and the regulation of the water homeostasis in all domains of life. These channels transport water and small molecules. Beyond the specificity of transport, their functional diversification would be determined both by the expression patterns and by the set of gating mechanisms and the interaction with proteins that regulate their location in the membrane, resulting in an alteration in water homeostasis.

Speakers

Victoria A Vitali (Postdoc CONICET - Instituto de Química y Fisicoquímica Biológicas "Prof. Alejandro C. Paladini", Argentina)

Functional diversification of aquaporins: the case of the PIP subfamily

María Florencia Leal Denis (Assistant Researcher CONICET - Instituto de Química y Fisicoquímica Biológicas " Prof. Alejandro C. Paladini", Argentina)

Effect of mastoran-7 and alpha-hemolysin on ATP release and cell volume in human erythrocytes. The role of aquaporin 1

Melisa Pucci Molineris (Postdoc CONICET - Instituto de Investigaciones Bioquímicas de La Plata "Prof. Dr. Rodolfo R. Brenner", Argentina)

Role of alpha-hemolysin in the extracellular matrix remodeling of human chorioamniotic membranes

YRS4 – Sarco-endoplasmic reticulum-mitochondrial coupling in physiology and pathophysiology

Coordinator: Julieta Palomeque (National University of La Plata & Independent Researcher, CONICET, Argentina)

The physical links between Sarco-endoplasmic reticulum (SR/ER) and mitochondria were first proposed over 40 years ago based on transmission electron microscopy of liver mitochondria. SR/ER and mitochondria join together at contact sites to form specific domains, termed mitochondria associated membranes (MAMs), with a characteristic set of proteins and distinct biochemical properties. Interorganellar contacts are increasingly recognized as central to the control of cellular behaviour. A significant body of evidence shows clearly that the association between SR/ER and mitochondria play important roles in several biological processes, e.g. ion and lipid transfer, inflammasome formation, unfolded protein response, autophagy, signalling and mitochondrial fission have been established.

Furthermore, the changes in MAMs have been implicated in different diseases, e.g. Alzheimer's disease, cancer, metabolic disease, and cardiac ischemia reperfusion. The objective of this symposium is to develop and highlight recent findings that reveal the crucial role of SR/ER-mitochondrial coupling in physiology and pathophysiology. We convoked 3 early careers from different countries and that have embraced this topic with enthusiasm. Although data available on the proteins that constitute MAMs are constantly increasing, there are still many uncertainties concerning the exact composition of these contact points and how it changes in response to various stimuli and cellular stress. The symposium is therefore more than timely, and the speakers proposed were selected to emphasize that SR/ER-mitochondria coupling has become a hot topic in physiology and pathophysiology.

Speakers

Marilén Federico (Cardiovascular Research Center, National University of La Plata (UNLP), Argentina)
SR-Mitochondria communication promotes mitochondrial damage and apoptosis in prediabetic hearts
Sergio De La Fuente Pérez (Thomas Jefferson University, USA)
Strategic positioning of the mitochondrial Ca2+ transporters at the SRmitochondria interface in the cardiac tissue
Roberto Bravo Sagua (Unidad de Nutrición Pública INTA - Universidad de Chile, Chile)
Caveolin-1 and PKA regulate ER-mitochondria communication during the early response to ER stress

YRS5 – Inflammation in the cardiovascular system: a multifaceted pathway
Coordinator: Verónica De Giusti (Universidad Nacional de La Plata & Adjunct Researcher, National Council for Scientific and Technical Research (CONICET), Argentina)
Although the role of inflammation in the onset of cardiovascular disease is not yet fully understood, inflammation is common in heart and vascular disease. It's important to know what inflammation is and how it can affect the cardiovascular system. This symposium covers traditional and non-traditional risk factors like hypertension, obesity, and air pollution exposure that have high prevalence in the modern society and are closely related to chronic inflammatory states that can injure the healthy heart by modulating specific intracellular signalling pathways. Although all molecular mechanisms have not been clearly defined, the exposure to pro inflammatory cytokines, reactive oxygen species and free fatty acids intermediaries have been suggested as key elements in the cardiovascular homeostasis. The study of the inflammatory role in these risk factors, as well as possible modulators, could gradually lead to development of more effective therapeutic strategies to prevent cardiovascular events.

Speakers

Rodrigo García (Doctoral Fellow, IMBECU-CONICET, Argentina)
Protective effects of hydroxychloroquine in cardiovascular remodelling associated with metabolic syndrome
Timoteo Marchini (Universidad de Buenos Aires, Argentina, Assistant Researcher IBIMOL-CONICET, Argentina & Researcher at the Friburgo University Hospital, Germany)
Air pollution particulate matter exposure aggravates myocardial infarction: The role of lung redox metabolism, inflammation and impaired cardiac mitochondrial function
Carolina Caniffi (Universidad de Buenos Aires, Argentina & Researcher IQUIMEFA-UBA-CONICET, Argentina)
Anti-inflammatory and anti-oxidant protection through C-type natriuretic peptide in normotension and hypertension

COMMUNITY OUTREACH ACTIVITY (COA)

COA1 – Why do I like to eat and other questions about eating and obesity (Por qué me gusta comer y otras preguntas sobre la alimentación y la obesidad). *This activity will be in Spanish.*

Coordinator: Claudio Pérez-Leighton. Pontificia Universidad Católica de Chile, Chile.

During the last decades, and despite the available treatments or policy interventions, obesity rates have steadily increased in Chile and worldwide. One of the main causes of obesity is excess food intake, which has been related to a lack of restrain in eating palatable, tasty food. However, our predilection for palatable food and our ability to eat it beyond satiety or without hunger is not just a problem of willpower, but it has deep biological roots that reach our brain and ability as a species to survive. In this seminar, we will discuss four questions about the scientific evidence about food intake and obesity: Why do we like to eat? What is the difference between hunger and desire to eat? What is an "edible" and is it different from food? and why do diets almost always fail? The goal of this seminar is to bring the science about eating and obesity closer to the general public to help them better understand their daily decisions about food and its health consequences.

COMPANY ACTIVITIES (CA)

CA1 – Portable laboratories for hybrid teaching in physiology (Laboratorios portátiles para enseñanza híbrida en fisiología). *This activity will be in Spanish.*

Coordinator: STALab+ (Chile)

This workshop will show how the innovations and use of online content platforms focused on Physiology have revolutionized practical laboratories, allowing portability and application in different educational models: distance, hybrid, inverted class, synchronous, asynchronous.

Speaker

Gabrielle Leite (ADInstruments)

Portable laboratories for hybrid teaching in physiology

YRS1 – Aquaporins in physiological and pathological conditions

Possible role of AQP3 in the etiology of preeclampsia

Natalia Szpilbarg[1]
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Preeclampsia affects 7–10% of pregnancies worldwide. It is characterized by hypertension and proteinuria after 20 weeks of gestation. The most severe form develops before 34 weeks of gestation and is associated with intrauterine growth restriction, alterations in trophoblast migration processes, and incomplete perfusion of the placenta. Furthermore, trophoblast apoptosis levels are elevated, leading to the development of clinical symptoms. Aquaporin-3 (AQP3) is expressed in human placenta from the onset to term gestation. Our objective was to study AQP3 expression in preeclamptic placentas and its role in processes related to the pathophysiology of preeclampsia, such as trophoblast migration in the first trimester and trophoblast apoptosis in the third trimester of pregnancy. Furthermore, we studied the possible association of AQP3 with caveolin-1 in the first trimester, since this protein is essential to generate the appropriate membrane domains for cell migration. This project was approved by the ethics committee of the Hospital Nacional ¨Prof. Dr. Alejandro Posadas¨ in Buenos Aires. AQP3 expression was analyzed in normal and preeclamptic term placentas. Additionally, an in vitro model of preeclampsia was used for apoptosis experiments in the third trimester. Finally, Swan 71 cell line was used for migration experiments and AQP3 and caveolin-1 association experiments in first trimester. The results showed that AQP3 is reduced in term preeclamptic placentas and that it participates in trophoblast migration and apoptosis in the first and third trimester respectively. Additionally, AQP3 colocalized with caveolin-1 in the first trimester, suggesting that this interaction may be necessary during placentation. In conclusion, further experiments are necessary to determine if the reduction of AQP3 comes from the beginning of pregnancy, being one possible cause of insufficient trophoblast migration in preeclampsia, or if it is a consequence of the increase in apoptotic levels as an attempt by the trophoblast to reduce damage. UBACyT 20020130200050 Universidad de Buenos Aires, Argentina

Role of Aquaporin-4 as an osmosensor in Retinal Müller cells: Implications in the physiopathology of Neuromyelitis Optica Spectrum Disorder

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Aquaporin-4 (AQP4) is the most abundant water channel expressed in the nervous system. Within the retina, AQP4 is mainly expressed in glial Müller cells, which control extracellular homeostasis. These cells regulate swelling, occurring because of the intense neuronal activity, by a regulatory volume decrease (RVD) mechanism which depends on the efflux of solutes and water through AQP4. AQP4 is also the target of autoantibody AQP4-IgG present in the sera of patients with Neuromyelitis Optica Spectrum Disorder (NMOSD), a severe demyelinating autoimmune disease. Müller cells respond to injury by re-entering the cell cycle for tissue repair. It was reported that AQP4 modulates cell volume during cell cycle progression to facilitate proliferation in astrocytes, but its involvement in Müller cells was not fully studied. In this work, we evaluated the role of AQP4 in Müller cells by two strategies: the use of the novel inhibitor TGN-020 and the binding of AQP4-IgG to AQP4 in the human Müller cell line MIO-M1. We measured cell volume, osmotic water permeability (Pf) and intracellular Ca2+ levels during hypotonic shock and cell proliferation. AQP4 inhibition by TGN-020 decreased Pf and RVD as expected, but also delayed hypotonicity-induced changes in Ca2+ kinetics, reinforcing the role of AQP4 as an osmosensor in Müller cells. These cell volume changes may be involved in cell proliferation, which was also reduced by TGN-020. AQP4-IgG positive sera from NMOSD patients decreased AQP4 plasma membrane expression, which was associated to a reduction in Pf, RVD and the magnitude of intracellular Ca2+ increase. Cell proliferation was also slower in comparison to control sera. We propose that AQP4 inhibition or removal from the plasma membrane reduces AQP4-mediated water permeability, altering cell proliferation. This is of particular importance in NMOSD, as the decreased ability of Müller cells to proliferate may affect retinal tissue repair in vivo.
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Hepatic gene transfer of Aquaporins for cholestasis

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Canalicular bile formation is an osmotic secretory process. The excretion of bile salts (BS), via the bile salt transporter BSEP/ABCB11 and organic anions (e.g. glutathione), via the organic anion transporter MRP2/ABCC2 are thought to be the major driving forces for the osmotic water movement into bile canaliculus via aquaporin-8 (AQP8) water channels. Canalicular AQP8 expression is defective in different rat models of cholestasis suggesting its involvement in bile secretory dysfunction. Our recent studies show that the hepatic gene delivery of human aquaporin-1 (haqp1), an archetypal AQP which transports water very efficiently, improves the bile secretory failure in estrogen-induced cholestatic rats by promoting biliary excretion and choleretic efficiency of BS [1,2]. We designed and performed studies to evaluate whether the administration of the AdhAQP1 vector promotes AQP1-mediated canalicular water secretion and improves hepatocyte bile secretory failure in a lipopolysaccharide (LPS)-induced cholestatic condition. AdhAQP1, administered by retrograde bile ductal infusion, induced hepatocyte canalicular hAQP1 expression. AdhAQP1 delivery normalized diminished bile flow, biliary BS [3] and glutathione [4] output in LPS-induced cholestasis. Moreover, markedly elevated serum BS levels in cholestatic rats, were almost restored with the AdhAQP1 hepatic transduction [3]. AdhAQP1-treatment unaffected the downregulated protein expression of canalicular BSEP/ABCB11or MRP2/ABCC2 in cholestasis [3, 4], but markedly increased their transport activities. As both transporter activities are critically dependent on membrane cholesterol content, the findings may be linked to the fact that hAQP1 expression restores reduced canalicular cholesterol content. Our results suggest that hAQP1-induced canalicular water permeability and BSEP/ABCC2 and MRP2/ABCC2 activation play a role in the improvement of LPS-induced cholestasis. This finding might contribute to new therapeutic approaches for endotoxin induced cholestatic diseases.References:1. Marrone et al. Gene Therapy 21:1058-64, 2014.2. Marrone et al. Hepatology 64:535-48, 2016.3. Marrone et al. IUBMB Life,69:978-984, 2017.4. Marrone et al. Biochimie ,165:179-182, 2019.
This work was supported by Grants PIP 2015-088 and PUE 0089 from CONICET, PICT 2015-0439 from ANPCyT to RAM and PICT2017-3393 from ANPCyT to JM.

YRS2 – Biology of exercise in metabolic disorders

Intracellular hydrogen peroxide as a signal for molecular responses to exercise

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Physical exercise is one of the most powerful interventions for preventing and treating chronic diseases. At the molecular level, there is mounting evidence that redox signaling by hydrogen peroxide (H2O2) could be involved in the myocellular adaptation to exercise. This study aimed to explore the changes in the major skeletal muscle peroxidases in response to acute exercise and long-term training. Peroxiredoxin (Prx) oxidation-dependent dimerization was monitored as a surrogate of H2O2 generation during in vitro and in vivo exercise in skeletal muscle cells. Acute electrical stimulation and in vivo exercise increased oxidation of cytosolic Prx(2) but not the mitochondrial Prx(3). A 6-week endurance training intervention increased both Prx2 and Prx3 content in mouse skeletal muscle and white adipose tissue. The training-induced upregulation of Prx2 was completed abolished in muscle-specific PGC1-alpha knockout mice. Interestingly, muscle-specific overexpression of PGC1-alpha was sufficient to increase Prx2/3 protein levels. Our results demonstrate that that acute exercise increases Prx2 oxidation and that exercise training increases H2O2 handling capacity by increasing Prx2 in a PGC1-alpha dependent manner.

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Novel insights into GLUT4 in insulin-sensitized and –resistant skeletal muscle

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Introduction: Insulin resistance (IR) is characterized by an impaired ability for insulin to stimulate glucose transporter (GLUT)4 translocation to the cell surface of muscle fibers to facilitate glucose uptake. Conversely, insulin sensitization (IS) by exercise occurs via increased GLUT4-associated muscle glucose uptake. The underlying mechanisms causing IR and IS are incompletely understood. Objectives: to investigate if microtubule-based GLUT4 distribution is impaired in muscle IR and to test if intramyocellular GLUT4 redistribution could be a mechanism for exercise-induced muscle IS. Methods: To study GLUT4 in IR we used structured illumination microscopy of endogenous GLUT4 and microtubules in human fibers from vastus lateralis muscle biopsies and mouse flexor digitorum brevis (FDB) muscle fibers. In addition, in FDB fibers we performed live-imaging of GLUT4 and the microtubules using genetically encoding fluorescent reporters. To study GLUT4 in IS we used sample thinning enhanced resolution microscopy to pinpoint GLUT4 to different compartments before and after insulin stimulation in human vastus lateralis biopsies obtained from resting and prior exercised muscle. All experiments were ethically approved according to institutional norms. Results: We found GLUT4 along the microtubules and at the microtubule nucleation sites in skeletal muscle fibers from mice and humans. In live

fibers, GLUT4 constitutively travelled on the microtubule filaments in the basal and insulin stimulated conditions. IR, induced by C2 ceramides or high fat diet, impaired microtubule-based GLUT4 trafficking. In humans, prior exercise induced a redistribution of GLUT4 to a compartment identified by the insulin- responsive-vesicle marker, VAMP2. Insulin stimulation of IS human muscle decreased the GLUT4 content in the VAMP2 positive compartment and increased the sarcolemmal and endosomal GLUT4 content compared to insulin stimulation of resting human muscle. Conclusions: Microtubule-based GLUT4 trafficking was impaired in IR muscle while exercise-induced IS was accompanied by intramyocellular GLUT4 redistribution.
The studies was funded by a Danish Diabetes Academy Research Grant, funded by the Novo Nordisk Foundation to Jonas Knudsen and a Novo Nordisk Excellence grant to Thomas Jensen.

Influence of exercise intensity on metabolic adaptations in an obesity context.
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Introduction: Obesity is associated with the development of metabolic disorders, particularly in insulin-sensitive tissues (e.g. skeletal muscle, adipose tissue, liver, heart). In that context, lifestyle modifications, such as exercise, has proved to be an effective strategy to counteract these disorders. However, if exercise programs at different intensities could exert differential metabolic effects on those tissues is unclear. Goal: To compare two exercise programs; constant-moderate endurance (END) and high intensity interval training (HIIT), in a mouse model of diet-induced obesity. Methodology: Male 10 week-old C57BL/6 mice were fed a high fat diet (HFD; 45% kcal fat) ad libitum for 10 weeks. For a further 10 weeks they underwent END or HIIT training (3 x 40 min sessions per week). Untrained HFD and chow-fed mice acted as controls. At termination, mice were sacrificed and quadriceps muscle, subcutaneous adipose tissue (SAT), liver, and heart were excised and analysed. Results: In quadriceps, HFD decreased high-molecular weight (HMW) adiponectin protein, which was normalized by END and HIIT. In SAT, only HIIT induced an increase in the mRNA (3-fold vs HFD untrained) and protein (2-fold vs HFD untrained) of UCP1. In liver, only END reversed collagen I accumulation seen in HFD untrained mice. In heart, HFD decreased HMW adiponectin protein, and only END reversed this change (2-fold vs HFD untrained).Conclusion. HFD was effective inducing metabolic disturbances in insulin sensitive tissues. Overall, exercise was effective by reversing these disturbances, however, specific effects from END and HIIT were seen. Future studies should explore the mechanisms behind these differences along with the clinical implications of these findings.
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YRS3 – Protein and membrane interaction, always a good interaction? Osmoionic imbalances and signalling mechanisms in pathological contexts

Functional diversification of aquaporins: the case of the PIP subfamily
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The membrane intrinsic proteins (MIP) family, better known as the aquaporin (AQP) family is a highly expanded group of channels. In eukaryotes, the best-known examples of MIP family expansion are land vertebrates, fishes and flowering plants. Previous phylogenetic studies revealed that each subfamily of plant AQPs was related to a subfamily of animal AQP predicting the presence of at least four families of AQPs in the ancestral eukaryote. We are particularly interested in the study of plant PIP (plasma membrane intrinsic proteins) subfamily that belongs to the AQP1-like group (also called classical aquaporins). This group includes not only animal AQP1 but also AQP0, AQP2, AQP4, AQP5 and AQP6. PIPs constitute the largest MIP subfamily (about 10 to 30 PIP genes are encoded by species) showing high sequence identity and two main groups of paralogous (PIP1 and PIP2) even in ancestral plants as Selaginella moellendorffii. For years efforts have been focused in elucidating whether this great multiplicity of isoforms implies diversity or functional overlap. Although a certain degree of redundancy is not ruled out, many pairs of duplicated genes show different expression patterns but functional diversity at the transport level has not been clear. The aim of our work is to analyse whether the high number of PIPs per plant presents genuine functional diversification. Here, we performed a comprehensive analysis of the PIP subfamily by combining coding sequences analysis, survey of tissue expression patterns, and characterization of biological activity. We described cases of functional diversification at solute specificity, cooperative response events and heterotetramer formation. Our results highlight the importance of multiple isoform conservation to describe the wide spectrum of biological activities. Deeping inside the PIP functional diversifications

could enlarge the spectrum of biological roles of other AQP belonging to the same group and could help to predict new functions.
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Effect of mastoran-7 and alpha-hemolysin on ATP release and cell volume in human erythrocytes. The role of aquaporin 1.

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Introduction: Human erythrocytes (hRBCs) release ATP in response to physio-pathophysiological. In vitro, exposure of hRBCs to the peptide MST7 (derived from wasp venom) and the toxin HlyA (derived from uropathogenic strains of E. coli), activates intracellular-signaling mechanisms leading to ATP release and cell swelling. Objective: We studied the regulation of cell volume (Vr) and extracellular ATP (ATPe) of hRBCs exposed to MST7 or HlyA. Additionally, we evaluated the effect of HlyA on water permeability through aquaporin 1 (AQP1), the main water-transport channel in hRBCs. Methodology: hRBCs from healthy donors and AQP1-KOs were exposed to MST7 or HlyA. ATPe was measured by luciferin-luciferase technique. Intracellular content of sodium (Na+i) and potassium (K+i) were measured by capillary electrophoresis and flame photometry. Changes in Vr were measure by fluorescence microscopy, the Coulter counting principle and by light scattering. Statistical significance was determined by one-way ANOVA followed by Turkey–Kramer test. The study was approved by Committee for Ethics on Clinical Investigation, FFyB, UBA (0048676/2017). Results: MST7 induced an increase of [ATPe] (500 %), Na+i (60%) and Vr (10%). Pharmacological inhibition of ATP release reduced swelling by 50%. Pre-treatment with P2X inhibitors reduced 48% [ATPe], 80% swelling and blocked Na+ uptake. The treatment of MST7 in hyperosmotic medium reduced 40% [ATPe] and blocked swelling. HlyA caused shrinking of hRBCs followed by continuous swelling, reaching 10% Vr increased over control values, while [ATPe] showed a 36-fold increased. Vr changes correlated with a 5-8 fold increase of [Na+i] and 5-7 fold decrease of [K+i]. HlyA decreased water permeability of hRBCs, both in WT and KO AQP1-hRBCs.ConclusionMST7- and HlyA- treated hRBCs triggered activation of ATP release, leading to P2X activation, followed by Na+ uptake, which in turn causes swelling. This increase in Vr caused further increments of ATP release, thus forming a positive feedback loop.
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Role of alpha-hemolysin in the extracellular matrix remodeling of human chorioamniotic membranes

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Introduction: α-hemolysin (HlyA), toxin secreted by uropathogenic strains of Escherichia coli, has a fundamental role in urinary tract infections (UTIs). In pregnancy UTIs are very frequent, being E. coli the etiological agent of almost the 80% of the cases. Considering that UTIs are related with premature rupture of fetal membranes, we proposed to analyze changes of human chorioamniotic membranes treated with HlyA in vitro. Methods: Chorioamniotic membranes (n=8) were obtained from deliveries by elective cesarean section (>37 weeks). All included women had normal pregnancies without evidence of active labor or infection. This protocol was approved by the Hospital Español Review Board (La Plata-EI001/19). Membrane explants were mounted and tied to a Transwell device generating two independent chambers. To simulate an ascending infection, explants were incubated in the chorion-side with 5nM/50nM HlyA during 24h. HlyA was detected by immunohistochemistry and histological signs of damage (like edema, vacuolization, and apoptosis) were evaluated from paraffin-embedded tissue sections stained with hematoxylin/eosin. Transepithelial electrical resistance (TEER) was measured using a Millicell-ERS unit, necrosis was evaluated by LDH release, (n=3), metalloprotease activity by zimography, and cyclooxygenase-2 (COX2) expression by RT-qPCR. Groups were compared using t-, U Mann-Whitney or Chi-squared test as correspond and values are shown as media ± SEM. Results: HlyA interaction with chorioamniotic membranes caused structural alterations and a slight diminish of TEER after 24hs of incubation. The main tissue alterations were observed for the highest toxin concentration tested (50nM HlyA). Epithelial layer remained practically unaltered, while chorion cells showed an increment of vacuolization and necrosis.

Extracellular matrix thickness, COX-2 expression and metalloproteinase activity were higher and fibroblast number lower in treated groups compared to control ones. Conclusion: HlyA by itself is capable to introduce structural and molecular modifications in human chorioamniotic membranes, suggesting a role of this toxin in chorioamniotic extracellular matrix remodeling.
Supported by Agencia Nacional de Promoción de Ciencia y Tecnología ANPCYT (PICT 2017-2393; PICT 2018-01400)-Argentina.

YRS4 – Sarco-endoplasmic reticulum-mitochondrial coupling in physiology and pathophysiology

SR-Mitochondria communication promotes mitochondrial damage and apoptosis in prediabetic hearts
Marilén Federico
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Separación espacial de los mecanismos de entrada y salida de Ca2+ mitocondrial en las conexiones retículo de cardiomiocitos adultos.
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Introduction: Control of myocardial energetics by Ca2+ signal propagation to the mitochondrial matrix includes local Ca2+ delivery from sarcoplasmic reticulum (SR) ryanodine receptors to the inner mitochondrial membrane, through the Ca2+ uniporter (mtCU) and it is extruded by the mitochondrial Na+/Ca2+ exchanger (NCLX). mtCU activity in cardiac mitochondria has been reported to be relatively low, hence, stochastically distributed mtCU and NCLX may not suffice to support local Ca2+ transfer and efficient mitochondrial Ca2+ signaling. Aims: The goal of the study is to elucidate whether the mitochondrial Ca2+ uptake and extrusion are physically separated within individual cardiac mitochondria, to enhance the Ca2+ signaling and optimizing, the membrane potential usage. Methods: Multiple approaches were used in the present study, including cellular and subcellular fractionation, super-resolution microscopy, mitochondrial Ca2+ measurements, size-exclusion chromatography and western-blotting. All studies were done following the NIH Guide for the Care and Use of Laboratory Animals, and the protocols were applied in compliance with the TJU guidelines. Data are presented as mean ±S.E. Statistical analysis was performed using Student's t-test. Results: mtCU distribution was biased toward the mitochondria-SR interface, and this bias was promoted by Ca2+ signaling activity in cardiomyocytes. The SR fraction of heart homogenate contains mitochondria with extensive SR associations, and these mitochondria are highly enriched in mtCU. Functional measurements suggested more effective mtCU-mediated Ca2+ uptake activity by the mitochondria of the SR than of the mitochondrial fraction. Our fractionation assays also reveal that extensively SR associated mitochondrial segments contain a minor portion of NCLX and lack of Na+-dependent Ca2+ extrusion. This pattern is retained upon NCLX overexpression, suggesting extensive targeting to non-SR-associated submitochondrial domains and functional relevance. Conclusions: In adult cardiac mitochondria the Ca2+ uptake and extrusion mechanisms are reciprocally polarized, to optimize the energy efficiency of local calcium signaling in the beating heart.
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Caveolin-1 and PKA antagonistically modulate mitochondrial metabolism through ER–mitochondria communication during early ER stress
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Introduction: Contact points between the endoplasmic reticulum (ER) and mitochondria enable Ca2+ transfer between both organelles, which boosts mitochondrial metabolism. During early ER stress, this communication increases as an adaptive mechanism. Aim: To characterize the signalling pathways controlling this response. We hypothesised that Caveolin-1 (CAV1) may be implicated, as it is enriched at ER–mitochondria contact sites. PKA was also a candidate, as its activity has been shown to regulate organelle dynamics. Methods: We used wild type HeLa cells or overexpressing CAV1. Early ER stress was induced with tunicamycin 0.5 µg/mL for 4 h. We measured ER–mitochondria contacts via electron microscopy and confocal microscopy. For Ca2+ transfer, we used the fluorescent probe Rhod-FF. To evaluate mitochondrial respiration, we measured a Clark's electrode. DRP1 phosphorylation was analysed through western blot. Cell viability was determined though annexin V staining using flow cytometry. All sample sizes were ≥ 3, data were

presented as mean ± sd, and analysed by 2-way ANOVA followed by Bonferroni post-test. Results: Early ER stress augmented ER–mitochondria contacts, which was prevented by CAV1 overexpression. This rendered ER-to-mitochondria Ca2+ transfer and mitochondrial bioenergetics unresponsive to ER stress. PKA inhibition with H89 or siRNA also impaired the increase in organelle contacts, Ca2+ transfer and mitochondrial respiration. CAV1 overexpression reduced PKA-mediated DRP1 phosphorylation, thereby enhancing ER stress-induced cell death. Increasing ER-mitochondria contacts with a synthetic linker restored cell survival. Conclusion: PKA promotes the increase of ER–mitochondria contacts that occurs during ER stress. CAV1, in turn, prevents PKA-mediated phosphorylation, also impairing said remodelling.
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YRS5 – Inflammation in the cardiovascular system: a multifaceted pathway

Air pollution particulate matter exposure aggravates myocardial infarction: The role of lung redox metabolism, inflammation and impaired cardiac mitochondrial function

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Introduction: Air pollution accounts for 2.4 million deaths from myocardial infarction (MI) every year. Fine particulate matter (PM2.5) – airborne particles < 2.5 µm in diameter that mainly arises from diesel exhaust in urban areas – has been pointed out as the main responsible. However, the underlying mechanisms are not completely understood. Aim: To evaluate the cardiorespiratory and systemic oxidative and inflammatory pathways triggered by PM2.5 inhalation in a combined model of continuous exposure to urban polluted air and experimental MI. Methods: BALB/c mice were exposed to filtered air (FA) or urban air (UA) inside whole-body inhalation chambers located in Buenos Aires City downtown (12 to 37 µg PM2.5/m3) during 16 weeks. Results: After 8 weeks, mice breathing UA showed a 56% increase in total leucocytes in bronchoalveolar lavage (BAL) samples (FA: 1.0±0.2 x105 cells, $p<0.05$) and a 104% increase in BAL protein concentration (FA: 0.30±0.04 mg/mL, $p<0.05$). Both were still increased after 12 weeks in UA-exposed mice, together with a 3-fold rise in MCP-1 levels. Lung leukocyte recruitment was confirmed by histology. Oxidative stress might precede inflammation, as increased pulmonary GSSG and decreased SOD activity, together with increased phospholipid oxidation, were found after 4 weeks. BAL analysis by flow cytometry showed alveolar macrophage accumulation and NO production in UA-exposed mice after 12 weeks. In this group, a significantly increased TNF-α and IL-6 plasma levels were also observed. At this time point, UA exposure induced a 53% increase in ischemia/reperfusion injury (FA: 43±4% risk area, $p<0.01$). Mechanistically, UA exposure lead to impaired cardiac mitochondrial function by decreased active respiration, inner membrane depolarization, increased H2O2 release, and decreased ATP production. Conclusion: Air pollution exposure induces a lung response that impairs cardiac mitochondrial function and worsens MI outcome. Our results highlight the importance of considering environmental factors in the development of cardiovascular diseases.
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Anti-inflammatory and anti-oxidant protection through C-type natriuretic peptide in normotension and hypertension

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Inflammation is a key mechanism in cardiac and vascular remodeling and dysfunction linked to hypertension. Moreover, inflammation exacerbates oxidative stress that, in turn, is associated with higher cardiac collagen deposition in hypertension. C-type natriuretic peptide (CNP) is an endothelium-derived factor with a cardiovascular protective role as previous studies from our and other groups suggested. We have previously shown that CNP attenuates the vascular damage development in a model of essential hypertension, inducing changes in fibrotic and inflammatory pathways that could contribute to beneficial effects on vascular morphology, extracellular matrix composition, and function. However, the in-vivo effect of CNP on cardiac remodeling linked to hypertension had not been investigated. As hypertension and cardiovascular diseases can be considered as a state of deficiency of natriuretic peptide effectiveness, we hypothesized that chronic CNP administration would have beneficial effects on cardiac remodeling by decreasing inflammation and fibrosis in hypertension. Therefore, we evaluated and compared the effects of chronic CNP administration on left ventricle remodeling and function in spontaneously hypertensive rats (SHR) and normotensive rats. We measured tumor necrosis factor-alpha, interleukin-1 and 6, transforming growth factor-beta 1, Smad proteins, nitric oxide synthase (NOS),

and the activity of superoxide dismutase, catalase, and glutathione peroxidase. Morphological studies were also performed. SHR showed signs of fibrosis and hypertrophy in left ventricle, higher NOS activity and more oxidative damage, as well as higher pro-inflammatory and pro-fibrotic markers than normotensive rats. Chronic CNP treatment attenuated hypertension and ventricular hypertrophy in SHR, with no changes in normotensive rats. In left ventricle, CNP decreased both pro-fibrotic and pro-inflammatory cytokines in SHR. In addition, CNP reduced oxidative damage as well as collagen content, and upregulated the NO system in both groups. Therefore, we concluded that chronic CNP treatment attenuates hypertension and associated heart damage by decreasing inflammation and fibrosis.
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Protective effects of hidroxichloroquine in cardiovascular remodeling associated with metabolic syndrome

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The cardiovascular remodeling process is an adaptive response to hemodynamic and inflammatory alterations that occur in hypertension, diabetes and / or chronic kidney disease. Thickening of the walls of large elastic and muscular arteries causes endothelial disfunction and increases the risk of cerebrovascular and coronary events. Previous clinical studies postulate that exist a relationship between low levels of C Reactive Protein (CRP) with reductions in major adverse cardiovascular events (MACE). Since the inflammation of the arterial wall plays a central role in the pathogenesis of atherosclerosis has led to the hypothesis that antiinflamatory or anticytokine therapies targeting specific interleukin signaling pathways could serve as powerful adjuncts to lipid lowering in the prevention and treatment of cardiovascular disease. Recently, canakinumab anti-inflammatory thrombosis outcomes study (CANTOS) has shown that specific targeting of Il-1β can significantly reduce cardiovascular event rates without lipid or blood pressure lowering. Hydroxychloroquine (HCQ) is an antimalaric and anti-rheumatic drug commonly used in the treatment of rheumatoid arthritis or systemic lupus erythematosus. In addition to its anti-inflammatory properties, different studies show that HCQ reduces cholesterol levels and the risk of type 2 diabetes, reduce glucose levels in diabetic patients, and has also antiplatelet effects. Evidence provided by the OXI trial and other smaller trials, as well as animal studies, in addition to the low cost of the drug, suggest that HCQ could be proposed as an entirely novel multitarget approach for the primary and secondary prevention of atherosclerotic and cardiovascular remodelling.

VIDEO POSTERS

Area: Education in Physiology

KQ691HT

Massive digital training for the management of the critically ill patient by COVID-19. Mexico 2020 experience.

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Introduction: The Mexican population has a high rate of comorbidities which condition a high risk of complications associated with COVID-19 infection. This pattern of prevalence and accelerated transmission generated a complex scenario for the containment of this pandemic, which urgently required highly trained personnel to provide timely medical care. Objective: To develop and evaluate the impact of an efficient digital mass training model to provide theoretical and practical knowledge for the management of critically ill patients with COVID-19. Method: An online educational intervention, designed for specialized medical personnel, was designed to care for the critically ill patient. A quantitative, cross-sectional, and descriptive study was carried out. Pre and post assessment materials were designed and applied to know the modification in medical knowledge through the intervention. Results: A cross-sectional, quantitative and descriptive study was carried out. 3016 health professionals, 29.7% men and 70.3% women, were trained, with an average age of 37.45 years (SD ± 11.2) years. The educational platform was satisfactorily evaluated by trained personnel, obtaining a score of 8.43 out of 10 (SD ± 1.66). Easy accessibility and applicability of the contents were demonstrated, as well as an adequate quality of didactic resources in the satisfaction survey. Normality of the sample's distribution in diagnostic and final test was found by using Shapiro-Wilk test. A statistically significant difference ($p < 0.0001$) between the means of diagnostic test and the final examination was found by using student's T test. Conclusions: Massive digital training is proposed as the main tool and the most accessible in time and cost to improve the knowledge of physicians about COVID-19 during the health contingency. Collaboration between non-profit institutions is possible and can lead to the development of valuable tools to improve medical training in Mexico.

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FT453DM

Perception of sepsis knowledge in different academic educational levels. 1 Morphophysiology Department, Medical Faculty, Universidad de La Sabana. Colombia.

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Introduction: Sepsis is considered a life-threatening organic disorder, which is secondary to a dysregulated response of the body to an infection. Incidence estimates report 19.4 million annual cases of sepsis and 5.3 million annual mortality due to sepsis, which makes it important to have an ample knowledge concerning sepsis, since it has been proven that the early identification and diagnostic approach is the main determinant of mortality in the septic patient. Objective(s): This study seeks to evaluate the general knowledge of sepsis and the insight regarding sepsis in different levels of medical training. Methodology: A quali-quantitative, observational descriptive study was performed based on a questionnaire in relation to the knowledge and the awareness of sepsis. 121 participants with different levels of education (medical students 7, medical interns 13, general physicians 44, social service physicians 11 and medical specialists 46) were evaluated. One-way Anova was used to perform the data analysis. This study wasn't submitted to an ethical committee as it did not imply an intervention, the personal data protection law (Habeas data) in Colombia (1581 of the 2012) was followed. Results: no statistically significant difference ($p=0.93$) between educational levels was evidenced. However, there was a statistically significant difference ($p<0.01$) between the phases of the knowledge of sepsis; overall, being the diagnostics the weakest and the treatment the utmost area of performance in the questionnaire. Conclusion(s): Despite the fact that the participants consider they comprehend the concept and the approach of the septic patient (80%), translating this to clinical practice, a lack of knowledge from all levels of academic education is unveiled (56%). This emphasizes the importance to reinforce the education in sepsis knowledge from basic medical sciences, especially physiology and pathophysiology, to optimize the management of septic patients in the emergency department.

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JD116CR

Virtual educational workshop: strategy for teaching, learning and evaluation of animal physiology at university level

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Introduction: An interactive teaching-learning strategy of the concept of thermoregulation is presented, considered a biological process of relevance in animal science; this strategy was carried out with students from the Animal Science curricular program of the National University of Colombia. Objective: A virtual workshop strategy is proposed, implemented and evaluated. Methodology: the virtual educational workshop was approached through the analysis and solution of a scientific problem on the subject of temperature control, recreating the behavior of the parameters of body temperature, heart rate, respiratory and urinary pH of domestic species such as cattle, sheep, goats, pigs and birds, exposed to two different environmental temperatures. The authors have presented the learning-teaching strategy, the method by which ten students approached the analysis and explanation of the problem and have also investigated those facts involved with the process of scientific conceptualization of the students around phenomena associated with thermoregulation, thermal equilibrium, heat esters and homeostasis. A qualitative analysis of the information was carried out to compare and evaluate the scientific conceptual change generated in the students between the beginning and the end of the course; In addition, a survey of studentsÂ´ perception of this educational methodology was carried out. Results: As the main result, it is indicated that the strategy implemented in the virtual workshop induced the scientific conceptual change, the ability to analyze and scientifically define a physiological phenomenon. Conclusion: collaborative work, the paradigm shift in the teacher-student relationship, enables meaningful learning and positive emotions that lead to motivation in the study of physiology. Keywords: didactics of science physiology, virtual education, problem approach, thermoregulation, conceptual change, emotions.

Area: Molecular and Cellular Physiology

CR717TS

Activation of adenosine receptors favors angiogenesis of endothelial progenitor cells

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Introduction: Endothelial Progenitor Cells (hEPC) are adult stem cells with the capacity for self-renewal and migration to neovascularization sites. Several stimuli promote hEPC recruitment, such as TNF-α and GM-CSF. Recent results from our laboratory show that activation of adenosine receptors increases migration and adhesion of hEPC to endothelial cells. However, the mechanism by which this nucleoside modulates the angiogenic capacity of hEPCs is unknown. Aims: it is to determine the contribution of VEGF and / or exosomes in the in vitro angiogenesis process of hEPC stimulated with adenosine. Method: The hEPC was extracted utilizing a density gradient and cultured for 3 days at 37°C and 5% CO2. The proliferation assays were performed by XTT assays. VEGF expression was determined by real-time PCR and dot-blot. Angiogenesis assays were performed on Matrigel and the isolation and characterization of exosomes were performed by ultracentrifugation, NTA, and flow cytometry, respectively. The Ethical Committee from the Facultad de Farmacia of Universidad de Concepción approved the protocol. Results: EPCs express VEGF and in the presence of NECA its expression and release increases. Furthermore, EPCs secrete exosomes into the extracellular medium with a size of less than 100 nm. The microvesicles express CD81, CD34, and KDR. Finally, the conditioned medium and exosomes favor the formation of capillary structures. Conclusion: These results suggest that the activation of adenosine receptors in EPC cells favors the release of VEGF and exosomes which could contribute to the formation of capillary-like structures.

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TB133PK

Activation of NLRP3 inflammasome decreases insulin-dependent GLUT4 translocation in skeletal muscle from obese mice

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Introduction: A cytosolic multiprotein complex called Inflammasome NLRP3 has been described as capable of mediate the release of interleukins in insulin-sensitive tissues, favoring the development of insulin resistance (IR). Available evidence suggests a relationship between impaired glucose metabolism and states of low-grade chronic inflammation in several tissues. IR is associated to impaired GLUT4 translocation, however, a direct effect of inflammasome NLRP3 activity on insulin-mediated GLUT4 translocation in skeletal muscle has not been clarified. Aim: To evaluate the expression of the NLRP3 inflammasome and its role on insulin-dependent Glut4 translocation in skeletal muscle from insulin resistance mice. Methods: Male C57BL/6 mice were fed with normal chow diet (NCD) or high fat diet (HFD) for 8 weeks. NLRP3 inflammasome components were analyzed by Western blot in homogenized of Flexor digitorium brevis (FDB) skeletal muscle. IL-1β plasma levels and caspase-1 activity were detected by ELISA test and a fluorometric assay, respectively. FDB muscle were transfected by electroporation with plasmid GLUT4myc-eGFP. All experiments were performed with n=3-9. Values were expressed as the mean ± SEM. Statistical significance was calculated using the Mann-Whitney test, and a value of $P \leq 0.05$ was considered statistically significant. The bioethics committee at Faculty of Medicine approved the protocols. Results: Compared to NCD-fed mice, both plasma levels of IL-1β and NLRP3 inflammasome components protein content in muscle homogenates were increased in HFD-fed mice. Interestingly, caspase-1 activity was also increased in skeletal muscle tissue of HFD-fed mice. In FDB fiber culture, the incubation of 10 µM MCC950 (a specific NLRP3 inflammasome inhibitor) resulted in a improve of insulin-dependent Glut4 translocation in both NCD and HFD-fed mice. Conclusion: NLRP3 inflammasome activity modulates insulin-dependent Glut4 translocation in skeletal muscle fibers. This process may be involved in the development of insulin resistance-induced by HFD in mice.

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JJ156CD

Golgi apparatus´ role in Immune Synapse formation in Natural Killer cells

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Introduction: Natural killer cells (NK) are the cytotoxic cells from the innate immune system. They form specialized junctions with target cells referred to as Immune Synapse (IS). IS is characterized by the local reorganization of actin and NK receptors, and the centrosome and Golgi apparatus (GA) translocation towards this site. In T cells, the GA regulates vesicle trafficking of signaling proteins that are essential for IS maturation. The GA role in NK-IS formation remained unexplored. AKAP350 is a centrosome and GA scaffold-protein that participates in the maintenance of GA integrity and in microtubule nucleation. Our previous results showed that AKAP350 knockdown impaired NK cytotoxic function and inhibited IS maturation. Objective: To evaluate the GA participation in IS maturation and AKAP350 relevance in this function. Methodology. YTS were used as NK cell-model, and KT86 as susceptible YTS targets. To interfere with the GA function, YTS cells were treated with brefeldin A (BFA). For analysis of YTS cytotoxicity, cells were incubated with CFSE-loaded KT86 cells for 4 hours, stained with Iodide Propidium and double positive events identified by FACS. Proteins clustering at the IS and localization was analyzed by immunofluorescence confocal microscopy. Results represent mean±s.e.m. in percentage. Paired t-student analysis was used. Results: BFA-treatment impaired YTS cytotoxic activity (control: 36%±3% BFA: 13%±2%*, n=3) and inhibited LFA-1 clustering at the IS (Control: 58%±5% BFA: 39%±4%*, n=16). AKAP350 localization at the GA increased during NK activation (+44%*). Displacement of AKAP350 from the GA by expression of its GA binding domain (GABD) impairs GA nucleation of microtubules and inhibited LFA-1 clustering (Control: 48%±4% GABD: 34%±3%*, n=16). (*p<0.05). Conclusion: Our results demonstrate that GA participates in NK-IS formation and that AKAP350 acts as a key regulatory protein in this process.

QL538PD

Differential fiber-type dependent gene expression modification of UPRmt in response to high fat diet-induced obesity.

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Introduction: Obesity is currently considered an epidemic due to the large world population it affects, its explosive growth and its association with metabolic disorders, such as insulin resistance. Skeletal muscle is the main tissue for glucose homeostasis in hyperglycemia, since it is responsible for approximately 80% of its insulin-induced uptake and catabolism. Previous studies mention a decrease in the amount of type I oxidative fibers and an increase in type II glycolytic fibers in this pathology. Molecular mechanisms underneath obesity and insulin resistance show that mitochondria have a key role. The Mitochondrial Unfolded Protein Response (UPRmt) is a recently describe adaptive mechanism that may underlie cell response to different types of stress and may be affected by high fat diet and obesity. Aims. To determine the changes in UPRmt related genes, such as mitochondrial protease ClpP and transcription factor CEBPb, in high fat diet-induced obese mice. Methods: Experimental animal protocols and procedures were approved by the Bioethics Committee for Research in Animals, Faculty of Medicine, University of Chile. Male C57BL/6J mice with a starting weight of 12-14 g were used, fed exclusively with a control diet (DC; 10% lipids, 20% proteins, 70% carbohydrates) or a high-fat diet (DAG; 60% lipids, 20 % protein, 20% carbohydrates) for 12 weeks (n = 7-8/group). The rapid tibialis anterior muscles and the slow soleus muscles were dissected. Gene expression of ClpP and CEBPb was assessed by Real time PCR. Data were analyzed using Student´s t-test for unpaired data (P<0.05) Results. Decreased mRNA levels of ClpP in HFD group compared to DC in Soleus muscle (slow oxidative) while increased CEBPb mRNA levels in HFD group compared to DC in tibialis anterior muscle (fast glycolytic) were found. Conclusions: UPRmt is differentially activated after high fat diet in obese mice in a fiber type dependent manner.
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LN933TC

Valproate increases the expression of Nephrin and Podocin and improves renal function in diabetic rats.

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Introduction: The expression of podocytes lineage specific genes Nephrin and Podocin is essential for the function and maintenance of the glomerular filtration barrier (GFB). In experimental models of diabetic nephropathy (DN), loss of histone H3 acetylation and enrichment of class IIa Histone Deacetylases (HDAC) enzymes in glomerular cells correlates with deposition of extracellular matrix and podocyte injury. Thus, inhibition of HDACs using the widely prescribed antiepileptic drug valproic acid (VPA) has been shown to downregulate fibrotic markers. Objective: We aim to evaluate if the beneficial effects of VPA are due to

effects on podocyte specific gene expression of Nephrin and Podocin. Methodology: Twelve Sprague-Dowley rats were divided in three groups: Control, diabetic and diabetic VPA treated rats. After induction of diabetes using streptozotocin metabolic proteinuria and glycemia were measured weekly. After 8 weeks of treatment, the rats were euthanized, and kidneys harvested for further study. This included measurement of acetylation levels and a-SMA using immunohistochemistry and the expression of Nephrin and Podocin. Statistically significant differences were estimated using Student's t-test with $P < 0.05$ with an $n = 4$. Results: VPA treatment reduced proteinuria and attenuated the fibrotic marker a-SMA in glomeruli when compared to diabetic rats. Nephrin and podocin expression were increased in purified glomeruli from diabetic rats treated with VPA, at both transcript and protein levels. Conclusion: VPA treatment improved kidney function parameters by recovering of the expression of Nephrin and Podocin. Acknowledgments: Funded by FONDECYT-Chile grants 1171340 (Rody San Martin) and 3170812 (Claudio Capelli).
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Search for pannexin homologues in pathogenic parasite Giardia sp.

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Introduction: In vertebrates, the channels formed by pannexin proteins play an important role in infectious diseases. However, homologs of these proteins have not been identified in unicellular pathogens. Giardia sp is the causative agent of giardiasis, a gastrointestinal disease that has a high prevalence worldwide and mainly affects children. The purpose of our research is to identify homologues of pannexin in Giardia sp. Methodology: The Giardia genome was searched using the BLASTP search facility of GiardiaDB hosted by the eukaryotic pathogen genomics database resource (EuPathDB). The protein topology was predicted using Protter software. For phylogenetic analysis, multiple sequence alignments were made with ClustalW (EMBL-EBI) using default parameters. Results: The genome of Giardia shows the existence of four genes that encoding homologues of pannexin proteins. The predicted protein products displaying significant similarity topology with protein of gap junction family with 4 transmembrane domains, 2 extracellular loops, 1 intracellular loop, and cytoplasmic C- and N-terminal domain. These homologues have an identity of 28.0% with human Panx-2 and 21.9% Caenorhabditis elegans Inx-1. Conclusions: These results suggest the presence of putative gap junction members in the protozoa parasite Giardia sp.
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ST852KH

Antifibrogenic effect of the angiotensin-(1-9) retroenantiomer in rat cardiac fibroblasts.

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Introduction: The development of cardiac fibrosis, orchestrated by cardiac fibroblasts (Cfs), is the excess deposition of extracellular matrix (i.e., collagen or fibronectin) in the cardiac muscle. We have previously shown that angiotensin-(1-9), a peptide of the renin-angiotensin contra-regulatory pathway, exerts anti-fibrotic in the heart. To increase its half-life, we generate a retro-enantiomer of angiotensin-(1-9) (RE). Whether this new peptide exerts anti-cardiac fibrogenic effects remains unexplored. Objectives: 1) to evaluate if RE prevents cardiac fibrogenesis triggered by TGF-β1. 2) To study whether RE interferes with the activation of TGF-β canonical pathway. Methodology: Primary cultures of neonatal rat CFs were pretreated or not for 1 h with angiotensin-(1-9) or R.E. before the addition of TGF-β. The protein levels of fibronectin, pro-collagen I, total-Smad3, and phospho-Smad3 were assessed by Western blot. Data are mean ± SEM, n=4-5. Statistical analysis was done ANOVA. The study was approved by the Bioethics Committee of Faculty of Chemical and Pharmaceutical Science. Results: The pre-treatment with RE or angiotensin-(1-9), but not the co-treatment, decreased fibronectin protein levels induced by exposure to TGF-β for 72 h. Phospho-Smad3 levels did not change by the treatment with any of both peptides. Conclusions. RE has a similar antifibrogenic effect than angiotensin-(1-9). However, RE does not interfere with the activation of the TGF-β canonical pathway in cultured cardiac fibroblasts.
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NP239CJ

Ligasa 1 de ubiquitina E3 mitocondrial (MUL1) en los efectos inducidos por palmitato sobre la desensibilización a la insulina y el metabolismo mitocondrial en mioblastos L6.

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Introduction: Circulating levels of free fatty acids are increased in obese patients, leading to its accumulation in skeletal muscle and the development of insulin resistance. MUL1 is a multifunctional ligase protein with an ubiquitin ligase E3 activity, which

participates in ubiquitin transfer cascade reactions. Both Akt and the mitochondrial fusion protein mitofusin-2 (MFN2) are regulated negatively by MUL1. Specifically, MUL1 ubiquitinates Akt and MFN2 causing their proteosomal degradation. MUL1 is increased after lipotoxic stress, but whether MUL1 participates in metabolic regulation and the insulin receptor signalling pathway is currently unknown. Aims: To study the role of MUL1 on insulin desensitization and mitochondrial metabolism in the L6 myoblasts subjected to lipotoxic stress. Methods: L6 myoblasts were treated with palmitate (12.5 nM) for 6 h and with a pulse of insulin (100 nM) in the last 15 min. The protein levels of total Akt, phospho-Akt, and MUL1 were analyzed by Western blot. Mitochondrial membrane potential and oxygen consumption were assessed by flow cytometry and Clark´s oxygraphy, respectively. A siRNA against MUL1 was used as an intervention tool. Data are shown as mean ± SEM of at least N=4. Data were analyzed by one or two-way ANOVA. Results: In L6 myoblasts, palmitate increased the protein levels of MUL1 and decreased the levels of p-Akt after the insulin pulse, which is consistent with insulin desensitization. Palmitate also decreased mitochondrial potential without changes in oxygen consumption. Using the siRNA for MUL1, we observed that the effects of palmitate on the insulin signalling pathway and mitochondrial potential were MUL1- dependent. Conclusion: These data suggest that MUL1 plays a key role in palmitate-induced insulin desensitization and in the maintenance of mitochondrial metabolism.
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BS295RP

Biomarkers of oxidative stress and inflammation in cardiovascular normorreactive and hyperreactive young adults

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Introduction: Cardiovascular hyperresponsiveness is the exaggerated response of the cardiovascular system to physical or mental stimuli, mainly evidenced by an increase in blood pressure and heart rate. It constitutes a predictor of arterial hypertension, which could be related to oxidative stress and low-grade systemic inflammation present in endothelial dysfunction. Objective: To determine oxidative and inflammation markers in normoreactive and hyperreactive cardiovascular young adults. Methodology: A descriptive cross-sectional study was conducted in 52 apparently healthy young adults, with an average of 19.85 ± 0,87 years old. The Isometric Test of Sustained Weight was performed to assess cardiovascular reactivity, 36 individuals were normoreactive and 16 hyperreactive. The activity of the enzymes superoxide dismutase, catalase and myeloperoxidase, as well as the concentrations of the products of the oxidation of lipids and proteins, were determined in serum. The values were expressed as mean ± standard deviation, the Mann-Whitney U test was used for the comparison between independent groups and the Spearman Rho correlation coefficient was used to determine the correlation between the variables. This research was approved by the Institution's Research Ethics Committee. Results: The hyperreactive group showed higher mean values of the protein oxidation products and the enzymatic activity of catalase, superoxide dismutase and myeloperoxidase, with significant differences in the mean values of myeloperoxidase when comparing both groups (0.424 ± 0.238 IU/ml vs 0.690 ± 0.396 IU/ml, p=0.004). A direct and highly significant correlation of myeloperoxidase activity with catalase was observed (Rho=0.366, p=0.008). Conclusion: Modifications in oxidative and inflammation biomarkers could reflect incipient alterations of an underlying low-grade inflammatory state and endothelial dysfunction in cardiovascular hyperreactive young adults, leading to an exaggerated pressor response to the isometric stress test.

CP387DP

Enzymatic activity of myeloperoxidase and its association with the pressor response to the Isometric Sustained Weight Test in young adults

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Introduction: Cardiovascular hyperresponsiveness is the exaggerated response of the cardiovascular system to a physical or mental stimulus, mainly evidenced by an increase in blood pressure and heart rate. Myeloperoxidase is one of the biomarkers of inflammation that is related to oxidative stress and endothelial dysfunction, so it could be involved in the pressor response to these stimuli. Objective: To determine the association between the enzymatic activity of myeloperoxidase and the blood pressure values obtained during the isometric stress test in young adults. Methodology: A descriptive cross-sectional study was carried out on 52 apparently healthy young adults, with an average of 19.85 ± 0,87 years old. The Isometric Sustained Weight Test was performed to assess cardiovascular reactivity and the enzymatic activity of myeloperoxidase was determined in serum by the o-Dianisidine method. Values were expressed as mean ± standard deviation and the Spearman Rho correlation coefficient was used to determine the correlation between the variables. This research was approved by the Institution's Research Ethics Committee. Results: The average value of myeloperoxidase activity was 0.507 ± 0.310 IU/ml. The activity of this enzyme showed weak and direct correlations with the blood pressure values measured two minutes after the isometric test, but not with the baseline blood pressure values. There was a correlation between myeloperoxidase activity with mean arterial pressure values (Rho=0.264, p=0.058) and a stronger and more significant correlation with diastolic pressure values (Rho=0.301, p=0.030).Conclusion: The higher enzyme activity of myeloperoxidase in young adults who presented higher values of diastolic blood pressure during the

isometric test suggests that this enzyme could be involved in the molecular basis of endothelial dysfunction that leads to a greater pressure response to isometric effort.

MG457GR

Role of RCAN1 in induced pluripotent stem cells (iPSC) and iPSC-derived cardiomyocytes from Down Syndrome patients

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Introduction: Down Syndrome (DS) is the most common autosomal aneuploidy, which is the product of an extra copy of chromosome 21 and is related to different neuronal and cardiac pathologies. DS patients present increased oxidative stress and, therefore, increased DNA damage; in addition to altered cell differentiation that would lead to failures in the organogenesis of these patients. In humans, RCAN1, located in the critical DS region of chromosome 21, is responsible of the enlarged and over functional mitochondria observed in DS (3S) iPSC. Objectives: To analyze the effect of RCAN1 on proliferation and DNA damage of 2S and 3S iPSCs, and to evaluate the role of this protein in the differentiation process of cardiomyocytes derived from these cells. Methodology: We measured the levels of the proliferation marker Ki67 and the DNA damage product 8-Oxoguanin. 2S and 3S iPSCs were differentiated into cardiomyocytes and the expression levels of stemness markers and cardiac genes were measured by q-RTPCR. The beating of iPSC-derived cardiomyocytes was analyzed before and after a Norepinephrine (NE) stimuli to assay functionality. As intervention tool, we used siRNAs for the two isoforms of the RCAN1 protein (1.1 and 1.4). Results were expressed as the mean ± SEM of at least N=3. Data were analyzed by Student t-Test or Two-way ANOVAs were applied. Results: RCAN1 overexpression in 3S iPSC induced an enhanced proliferation and cumulative DNA damage, which is dependent on the expression levels of the RCAN1.1 isoform. 3S iPSC-derived cardiomyocytes expressed lower levels of cardiac differentiation markers than 2S cells after 20 days of culture. Additionally, these cells do not respond to NE, although their basal beating rate is higher. Conclusion: RCAN1 overexpression regulates the increased proliferation and DNA damage observed in 3S iPSC; together with a decrease in the 3S iPSC differentiation ability towards a cardiomyocyte lineage.

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HR228FB

Sucralose consumption improves liver metabolism in mice fed a high-fat diet

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Introduction: Nonalcoholic fatty liver disease (NAFLD) is an accumulation of intrahepatic triglycerides associated with obesity. As a measure to face obesity and related diseases, Chile implemented the labeling of critical nutrients excess in food, which produces a change in preferences for low-calorie foods, such as sucralose, a non-caloric sweetener. However, there are controversial data about the effect of sucralose in health. Objective: To determine the effect of sucralose in the development of NAFLD. Methodology: Male C57BL / 6 mice were fed a control diet (DC) or high fat diet (HFD) for 8 weeks and supplemented with sucralose in water (0.1 mg / ml). In addition to body and liver weight and food / beverage intake, we measured biochemical parameters, adipose tissue, and glucose homeostasis using glucose and pyruvate tolerance tests. Triglycerides and mitochondrial respiration rate in the liver were also evaluated. Also, mitochondrial protein markers and proteins involved in gluconeogenesis and lipogenesis were evaluated by western blot in the liver. Data are presented as mean ± standard error of the mean and statistical significance was determined through two-way ANOVA (n = 10-13) .This project was approved by the Institutional Committee for Care and Use of Animals (CICUA) of the University of Chile. Results: Sucralose supplementation did not affect food or drink intake or the body weight and adipose tissue gain during the 8-week treatment. However, it improved glucose tolerance and prevented the decrease in mitochondrial mass in mice fed HFD, with no effect on mitochondrial biogenesis, liver weight or mitochondrial respiration rate. Finally, sucralose also did not alter the levels of gluconeogenic or lipogenic enzymes. Conclusions: These results suggest that sucralose has a beneficial effect on metabolism in animals fed with HFD; however, the mechanisms are still unknown.

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GS645KP

Masseter muscle paralysis evoked by Botulinum Toxin Type A exacerbates the signaling pathway of extracellular ATP in mice

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Introduction: Extracellular ATP (eATP) is relevant for skeletal muscle activity and plasticity. However, a deregulated release of ATP has been associated with muscle damage in animal models of hindlimb paralysis, muscle dystrophy, and aging. Nowadays, the paralysis of masticatory muscles by Botulinum Toxin Type A (BoNTA) injections is widely used for aesthetic and therapeutic approaches. However, the status of the eATP signaling pathway has been never addressed in masticatory muscles and even less after BoNTA-evoked paralysis. Aim: To assess whether masseter muscle paralysis evoked by BoNTA exacerbates the eATP-signaling pathway in mouse. Methods: 8 weeks-old male mice were injected in the right masseter muscle with 0,2U/10µl BoNTA, and in the left masseter with saline solution, as approved by the IACUC of the Universidad de Chile (17011-OD-UCH). After 2, 7, or 14d, muscles were dissected to analyze ATP release (luciferin-luciferase reaction). Expression of mRNA levels (qRT-PCR) or protein detection (immunofluorescence) of molecules related to ATP release (connexins, pannexins) or activity (P2X/P2Y receptor) were addressed, as well as the reactivity to masseter muscles to exogenous ATP in vitro. Data was expressed as Mean±SEM; Mann-Whitney test was used for comparisons (n=3-5).Results: A 50%-increase in eATP resting levels was observed in masseter muscles after BoNTA injection, compared with the contralateral saline-injected side. BoNTA injected muscles also showed increased mRNA and protein levels of hemichannel-forming molecules (Pannexin1, Connexin43-45) as well as eATP-receptors subtypes (P2Y2, P2X7). Moreover, the dose-response to exogenous ATP in vitro was obliterated in masseter muscles injected with BoNTA. Conclusion: BoNTA-evoked paralysis of masseter muscles increases the ATP release, as well as the relative levels of mRNA coding for several molecular components of the eATP signaling pathway, which modifies the magnitude of its effects.
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FC871CR

17-β estradiol regulates MUL1 and hypertrophy in cultured rat cardiomyocytes

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Introduction: Cardiovascular disease risk is higher in men than in premenopausal women of the same age, but this female advantage is lost after menopause. This observation has led to ask whether decreased estrogen (E2) synthesis could be associated with the development of cardiac hypertrophy (HC), a process characterized by increases in cardiomyocyte size, protein synthesis and in the re-expression of the cardiac fetal gene program together with a decreased cardiac oxidative energy metabolism. High protein levels of the mitochondrial protein ubiquitin E3 ligase 1 (MUL1) have been found in the heart. MUL1 acts preferentially as a SUMO E3, but it also catalyzes the ubiquitination of several target proteins, including the mitochondrial fusion protein, mitofusin. Therefore, MUL1 affects the dynamic balance between mitochondrial fission and fusion by promoting mitochondrial fragmentation, which is enough to produce HC. Aims: To in vitro study the effects of E2 on cardiomyocyte hypertrophy and to investigate whether E2 prevents the increases in MUL1 protein levels observed in hypertrophied cardiomyocytes. Methods and results. Cultures of neonatal rat ventricular myocytes (NRVM) were preincubated with or without E2 prior to the treatment with norepinephrine (NE) 10 mM for 48 h. NE increases the protein levels of the hypertrophy marker ANP, and MUL1 assessed by RT-qPCR and Western blot, respectively; as well as cardiomyocyte area and mitochondrial fragmentation. All these parameters were decreased with the pre-treatment with E2. Data are shown as mean ± SEM and represent experiments performed on at least four different occasions. Data were analyzed by one-way ANOVA. Conclusions: In an in vitro model of NRVM, E2 decreases cardiomyocyte hypertrophy markers and prevents the increase in the protein levels of MUL1 and the mitochondrial fragmentation triggered by NE. However, it remains to investigate the molecular link between these findings.
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FD298JC

IL-6 and myostatin expression in mouse aorta during endurance exercise

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Introduction: Skeletal muscle secretes myokines that coordinate muscle activity with other biological systems to meet the body's energy requirement during exercise. Interleukin-6 (IL-6) is a myokine that increases its mRNA levels at the end of the exercise session and regulates metabolic functions and muscle growth. Myostatin is a myokine that, after physical activity, decreases its mRNA levels and negatively regulates muscle growth. High-performance athletes undergo vascular remodeling as adaptation to physical activity. The expression of IL-6 and Myostatin has been demonstrated in rat aortic smooth muscle cells, but it is unknown

whether their expression is regulated by exercise. Objective: The objective of this work is to determine whether acute endurance exercise regulates the expression of these myokines in mouse aorta. Methods: C57BL6 mice ran once for 0, 30, 60, and 90 minutes at 60% of their maximum aerobic speed (MAS). Mice were euthanized immediately after finished the exercise session and their aorta and quadriceps were removed. The mRNA of both samples tissue was analyzed by RT-qPCR. Institutional bioethical committee approved this protocol. Results: The results showed a significant difference in MAS between control and 90-minute groups. Both myokines can be detected in mouse aorta by the action of exercise. Contrary to our hypothesis, Myostatin also showed an increasing trend in both tissues. Conclusion: It was concluded that although the data are preliminary, the results are very promising to continue exploring the possibility that exercise induces the expression of these myokines in mouse aorta.
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BB775FN

Maternal supraphysiological hypercholesterolemia associates with increased levels of lipid peroxidation and susceptibility to oxidation of maternal LDL, along with increased antioxidant capacity of HDL.

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Introduction: Maternal physiological hypercholesterolemia (MPH; total cholesterol (TC) ≤280 mg/dL) occurs during pregnancy. However, some women develop maternal supraphysiological hypercholesterolemia (MSPH; TC>280 mg/dL) which associates with fetoplacental endothelial dysfunction and atherosclerosis. MSPH could not only modify maternal cholesterol levels, but also the composition and function of lipoproteins, like its oxidative status, contributing to cardiovascular disease. Aim: To determine the oxidative status of maternal serum and lipoproteins in MPH and MSPH pregnant women. Methods: Maternal serum from MPH (n=43) and MSPH (n=28) women were collected at term of pregnancy. Lipid profile was determined in the samples. Maternal LDL and HDL were purified by ultracentrifugation. Lipid peroxidation was estimated as malondialdehyde (MDA) concentration in the maternal serum. Lipoprotein pro/anti-oxidant capacity was determined by reactive oxygen species (ROS) quantification (dichlorofluorescein probe) and by conjugated dienes. Values are mean ± standard error mean. This study was approved by the Ethics Committee PUC. Results: TC and LDL were increased in MSPH compare to MPH serum (39.7±5.9% and 66.4±5.8%, respectively). MDA levels were higher in MSPH compared to MPH serum (18,9±0,8 v/s 13,7±1,6 µM MDA, respectively). When ROS levels were measured, LDL from MSPH showed increased pro-oxidant activity (43.8±7.8%) and HDL an increased antioxidant capacity (28.7±3.5%) compare to MPH. Oxidation curves of the conjugated dienes assay showed that LDL from MSPH have increased susceptibility to oxidation as shown by reduced time of lag phase compared to MPH (56.1±1.8 v/s 73.4±1.7 minutes, respectively). Conclusion: MSPH associates not only with changes in maternal cholesterol levels, but also with changes in the oxidative status of maternal serum and its lipoproteins. Increased maternal HDL antioxidant capacity may be to counteract the susceptibility to oxidation and the pro-oxidative environment provided by LDL from MSPH pregnant women.
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PK247LD

Role of autophagy and oxidized LDL during first trimester trophoblast migration, invasion and differentiation

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Introduction: Placentation during the first trimester of pregnancy requires that the trophoblastic cells invade the maternal arteries and differentiate towards an endothelial phenotype. Oxidized LDL (ox-LDL) impairs trophoblast invasion in vitro and changes basal autophagy rate in different cellular models. However, the role of autophagy and its modulation by ox-LDL in trophoblast cell function is unknown. Aim: To determine if autophagy is involved in trophoblast migration, invasion, and differentiation, and assess the effect of ox-LDL in these processes and on the autophagic flux. Methods: The first trimester trophoblast cell line HTR8/SVneo was used to determine autophagy markers by western blot (LC3I and II, Beclin-1, ATG7, and p62), migration (transwell assay), invasion (transwell with matrigel assay), and trophoblast to endothelial differentiation in Matrigel matrix (tube formation assays and determination of endothelial marker ve-cadherin) in presence or absence of the autophagy inhibitor (bafilomycin A1, 50nM) and/or ox-LDL (50- 100 ?g/mL, 6h). LDL was isolated from adult donors and oxidized with CuSO4. Results: HTR8/SVneo were differentiated to endothelial like cells. The protein levels of LC3 decreased in 99% and p62 in 61%. The autophagy inhibition led to increased LC3 (2,4 folds) and p62 (2,6 folds), and the differentiation was reduced. To assay the effects of ox-LDL on autophagy, cells were exposed to ox-LDL, which modified ATG7 (0,47±0 vs 0,24±0 AU) and p62 (0,29±0 vs 17±0 AU) protein levels compared to control cells. LC3 and Beclin-1 were unaltered. Migration and invasion were reduced in 60% and 50%, respectively, in cells exposed to ox-LDL compared to control cells. Conclusion: Autophagy participates in the vascular remodeling process in trophoblast cells, and ox-LDL could be a factor affecting autophagy and, therefore, placentation. Future studies are required to determine the role of autophagy and placentation.
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JC615CJ

AP1 activity is required for arsenic trioxide-increased MDCK cells proliferation

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Introduction: Arsenic trioxide (ATO) increased the Na+/H+ exchanger 1 (NHE1) expression and activity, resulting in intracellular alkalization and higher MDCK cells proliferation. Acidic intracellular pH (pHi) activates the pro-proliferative transcription factor activator protein 1 (AP-1); however, its role in MDCK cells proliferation is unknown. Objective: we asked whether ATO-increased MDCK cells proliferation involves AP-1–dependent NHE1 activation. Methods: Cells were exposed (48 h) to ATO (0.05 mmol/L), SR11302 (1 mmol/L, AP-1 inhibitor), HOE-694 (100 nmol/L, NHE1 inhibitor) and EIPA (50 mmol/L, NHE1/NHE3 inhibitor) in the presence of S3226 (10 mmol/L, NHE3 inhibitor), concanamycin A (0.1 mmol/L, V-ATPases inhibitor), and Schering (10 mmol /L, H+/K+-ATPase inhibitor). [3H]Thymidine incorporation, cell counting (haemocytometer), wound healing assay, and AP-1 activity were determined. The pHi was measured in cells pre-loaded (10 min) with the fluorescent pH-sensitive probe BCECF-AM (12 µmol/L) and exposed to NH4Cl (20 mmol/L). Basal pHi and recovery rate (dpHi/dt), intracellular buffer capacity (bi) and H+ flux (JH+) were determined. NHE1 protein abundance was measured by Western blotting and immunofluorescence. Results. ATO increased the cell growth (1.5 fold) (one-way ANOVA), basal pHi (0.4 pHi units), dpHi/dt (1.8 fold), JH+ (1.4 fold), AP-1 activity and NHE1 protein abundance (1.3 fold). ATO also increased (1.5 fold) the nuclear/perinuclear NHE1 immunosignal. SR11302 and HOE-694 blocked ATO effects. Conclusion: ATO-increased proliferation resulted from AP-1–dependent NHE1 activation in MDCK cells.

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CP799DJ

Use of phospholipid nanomicelles for Membrane Lipid Replacement in damaged membranes.

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Introduction: Different processes can disruptcell membranes, leading to loss of cell homeostasis causing cell death. Here, we tested in different cells under membrane stress situations, if Membrane Lipid Replacement (MLR) with nanomicelles can avoid or slow membrane disarrangements. Objective: To test if MLR with nanomicelles made of glycerophospholipid mixtures (GPL) can increase cell function or viability in situations where membrane stress plays a leading role. Methodology: Human sperm cells and isolated cardiomyocytes (guinea-pigs) were used. Protocols were approved by the National Ethics Committee (School of Medicine, UdelaR). Fresh Sperm was obtained after ejaculation by a standard swim-up protocolor by thawing cryopreserved samples. Isolated cardiomyocytes were obtained through enzymatic protocols. Nanomicelles were obtained through ultrasonication at 10 KHz (>10 min). Dose-Response curves resulted from hydrogen peroxide (H2O2) exposure at different concentrations (>1 hour), using either untreated or treated cells with 0.1% nanomicelles. Analysis was done with Sigmaplot. A Hill model was fitted to dose-response data with non-linear regression. Results are expressed as mean ± s.e.m (n=4).Results. After incubation with H2O2 alone or combined with 0.1% nanomicelles: a) The motility of fresh sperm cells improved after exposure to increasing concentrations of H2O2(IC50=92±14 and 743±68 µM), b) Mitochondrial membrane potential after damage by H2O2 was maintained (JC-1 ratio red/green 0.27±0.08 to 8.4±0.8), c) Cryopreserved sperm cells thawed had more vitality (eosin-negrosin, IC50 4±1.2 to 88±12 %), and d) ventricular cardiomyocytes were more resistant to Ca2+ overload (IC50 82±12 to 342±42µM).Conclusions. Nanomicelles fuse with membranes restoring damage to membrane phospholipidswhile recoveringcell vital functions and restored mitochondrial function. Thus, MLR is a useful approach to treat or prevent processes in which cell membranes are damaged by oxidants.

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FQ774PK

The specific inhibition of the cardiac electrogenic sodium/bicarbonate cotransporter leads to cardiac hypertrophy.

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Introduction: The Na+/HCO3- cotransporter (NBC) is one of the main alkalinizing transporters of the cardiomyocytes. There are two isoforms of NBC: the electrogenic NBCe1 and the electroneutral NBCn1. Although both isoforms enters Na+ into the cell, NBCe1 contributes with half of Na+ per HCO3-, evidencing a better efficiency. We have previously found a reduction of NBCe1 activity together with an increased NBCn1 activity in cardiac hypertrophy (CH) models. Aims: We developed an interference RNA cloned in a cardiotropic adeno-associated vector (AAV9-shNBCe1) to study the effect of the specific inhibition of NBCe1 in CH. Methodology: We delivered the virus through a lateral tail vein injection in 3 months old male Wistar rats and then performed a series of studies to assess CH, using an AAV9-shControl as control. Data were expressed as means±S.E.M. and compared with Student's t-test or two-way ANOVA. The experimental protocol was approved by the Animal Welfare Committee of La Plata School of Medicine. Results: After 30 days of injection, we confirm a significant reduction on NBCe1 ventricular expression. In addition, we found an increase in left ventricular mass index obtained by echocardiography on hearts injected with AAV9-shNBCe1 (AAV9-shControl: 1.01±0.1; n=11; AAV9-shNBCe1*: 1.46±0.11, n=11; *p<0.05 vs AAV9-shControl). This result was consistent with cardiomyocytes' cross-sectional area analysis. No differences were found in blood pressure. Furthermore, some preliminary results indicate a compensatory increase in NBCn1 and Na+/H+ exchanger expression. Conclusion: Overall, these results suggest that the CH is developed, at least in part, by the decrease in NBCe1 expression. We propose that this reduction triggers a compensatory response involving the increase in the expression and activity of the remaining alkalinizing transporters. This mechanism would in turn induce the enhancement of intracellular Na+ levels, leading to Ca+2 overload trough Na+/ Ca+2 exchanger acting in its reverse mode. Such increase of Ca+2 could lead to CH.

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KR793NF

HIF-2α through A2BAR regulates invasiveness capacity of Glioblastoma Stem-like cells under hypoxic conditions

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Introduction: Glioblastoma (GBM) is the most common and deadly malignant brain tumor, with a patient´s median survival rate from 15 to 17 months. GBM contains a cellular subpopulation known as Glioblastoma stem-like cells (GSCs) that persists in hypoxic niches. These cells are capable to infiltrate into healthy brain tissue and are considered as the responsible of tumor recurrence. Hypoxia stabilizes HIF-2α and increases A2BAR expression, considering as central player of cancer cells adaptation to the hypoxic microenvironment. HIF-2α and A2BAR are capable to modulate the aggressiveness of different cancer models, however, the role of these two proteins in the invasiveness of GSCs under hypoxic conditions, is still unknown. Objective: to understand the role of HIF-2α and A2BAR in modulating migratory/invasive capacity of GSCs under hypoxia. Methodology: A2BAR and HIF-2α expression in GBM tissue was evaluated in silico in The Cancer Genome Atlas (TCGA) database. GSCs derived from U87MG cell line (GSCs-U87MG) and primary culture (GSCs-PC) were cultured under normoxia (21% O2) and hypoxia (0.5% O2). MRS1754 was used as A2BAR antagonist and specific siRNA for HIF-2α knockdown. mRNA levels of TWIST1, SNAIL, MMP9, HIF-2α and A2BAR were evaluated by RT-qPCR. The migratory and invasive capacity were evaluated by transwell and transwell-matrigel assays, respectively. All data were presented as mean ± standard deviation and analyzed with GradphPad Software. The mean values of two groups were compared by Student's t test. Results. A2BAR expression was associated with GBM tissue and necrotic areas. Migratory and invasive capacity of GSCs increased under hypoxic conditions. A2BAR blockage decreased the invasiveness capacity of GSCs, downregulating MMP9, SNAIL and TWIST1 expression, and similar results were obtained with knockdown of HIF-2α. Additionally, knockdown of HIF-2α reduced A2BAR expression. Conclusions. HIF2α through A2BAR signaling, regulates the invasiveness phenotype of GSCs under hypoxic conditions.

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JH971MJ

The non-canonical Notch pathway regulate the mesenchymal to endothelial-like transition of human extravillus trophoblast cells

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Introduction: Extravillous trophoblast (EVT)-dependent remodelling of the uterine arteries during placental development relay on the EVTs differentiation to an endothelial-like phenotype by a mesenchymal to endothelial-like transition (MELT). Defects in this process associates to severe gestational syndrome such as preeclampsia. Thus, elucidate the exact regulatory mechanism of MELT

result necessary. Notch pathway regulate proliferation, cell fate and differentiation processes by the activation of the Notch intracellular domain (NICD), triggering the activation of the canonical pathway (NICD translocation to the nucleus) and/or the non-canonical pathways (Cytosolic NICD). Aim: To determine if the canonical and/or non-canonical Notch pathway regulate MELT in EVTs. Methods: MELT were studied in the EVTs cell line, HTR8/SVneo cells, by a matrigel-tube formation assay. Angiogenic parameters were analyzed by ImageJ software. Cell extracts from MELT cultures were analyzed for NICD activation and Notch targets gene expression by western blot. Subcellular fractionation were developed to evaluate the nucleus/cytoplasm (N/C) distribution of NICD. Activation of the canonical Notch pathway was determined by reporter assays. This project has the approval of the Ethics and Biosafety committee of the Universidad San Sebastian, period 2018 to 2022. Results: The protein abundance of N1ICD and N2ICD increased (3 ± 0,1 fold and 2,5 ± 0,2 fold respectively) while the N/C ratio of N1ICD (6 ± 0,1 fold) and the canonical Notch pathway activity decrees (10 ± 0,1 fold) during MELT. Conclusion: Our results suggest a switch from canonical to non-canonical Notch pathway during MELT proposing the non-canonical Notch pathway as a key regulator of this process.
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PM238BS

Cardiotoxic effects of chemotherapy medication (5-FluoroUracil and Cisplatin)

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Introduction: Chemotherapy medication exhibits cardiotoxicity. 5-Fluorouracil (5-FU) and cisplatin (CPT), are used alone or combined in various cancer treatments (i.e. 5-FU+CPT, gastrointestinal cancers). This work starts characterizing their cardiotoxicity in isolated hearts and cells. Objectives: To characterize the impairment of cardiac performance in isolated hearts and cardiomyocytes from guinea pigs (Cavia porcellus) due to acute exposure to 5-FU and CPT towards understanding their mechanisms of induced cardiotoxicity. Methods: All the procedures were performed following protocols submitted to and approved by "Comisión Honoraria de Experimentación Animal" (Exp # 070153-000118-17). Isolated hearts were placed on the Langendorff system and perfused with Tyrode 1.8 mM Ca2+ alone or with the addition of 5-FU (0-600 µM) and/or CPT (0-100µM). The strain was recorded through a transducer in the base of the papillary muscle. Electrical responses were measured with Ag-AgCl electrodes next to the papillary muscle. Cardiomyocytes were isolated by enzymatic methods [3]. Data were obtained by Axon products. Confocal microscopy was done with Rhodamine/Fluo for Ca2+. Statistic tests between treated and not treated were not parametric (Mann-Whitney-Wilcoxon). The best fit of Hill´s equation to dose-response curves used nonlinear regression. Results are shown as mean +/- s.e.m (n=3). Results: In isolated hearts, 5-FU decreased the heart rate (IC50 =414±37 µM). It also had a negative inotropic effect (IC50=284±28 µM) and it increased the rate of irregular beats over 10% per 30 seconds/bin, above 400 µM. In isolated cardiomyocytes, spontaneous Ca2+ waves, intracellular Ca2+ diminished. Adding CPT 3 µM diminished the effects observed with 5-FU alone in isolated hearts (IC50=610±48 µM, 394 ±39 µM and 600 µM) and cardiomyocytes (intracellular Ca2+). Conclusions: Though 5-FU and CPT have cardiotoxicity, their mechanisms seem to be different. Its combination seems to cause less cardiotoxicity that their administration alone.

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RG352SB

Efecto inhibidor de nuevos acrilonitrilos sobre la actividad Nox4 en células de músculo liso vascular.

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Introduction: In cardiovascular disease, such as atherosclerosis and hypertension, differentiation of vascular smooth muscle cells (VSMC) is regulated by changes in the levels of reactive oxygen species (ROS). ROS production by NADPH oxidase 4 (NOX4) is stimulated by TGF-ß during the process of differentiation of VSMC. TGF-ß through NOX4-mediated ROS production regulates signal transduction pathways that involve activation of the Akt and SMAD, that finally leads to the expression of factors needed for the differentiation of VSMC, such as SM22, ?-SMA and Hic-5. Furthermore, our group has produced a series of heterocyclic compounds (acrylonitriles), that have shown inhibitory activity on NOXs, namely P1 ((E)-2-(1H-indole-3-carbonyl)-3-(pyridin-4-yl) acrylonitrile) and P3 ((E)-2-(1H-indole-3-carbonyl)-3-(5-phenylisoxazol-3-yl) acrylonitrile). Objective: To test the hypothesis that acrylonitriles inhibit NOX4 ROS production, interfering with VSMC differentiation. Methodology: Human VSMCs were stimulated or not with TGF-ß (4 ng/mL, 24 hours) in the presence or absence of P1 and P3 (10 µmol/L, 4 hours). Intracellular ROS levels were evaluated using the Amplex-red technique. Protein expression and

phosphorylation was evaluated by Western-blotting. Data was analyzed by one-way ANOVA, n=3. The protocol was approved by the ethical committee of Emory University. Results: Acrylonitriles P1 and P3 inhibited the production of ROS stimulated by TGF-ß (p=0.0001; p=0.0002 respectively). P1 and P3 reduced the levels of phospho-Akt (p=0.002; p=0.0072 respectively) and phospho-SMAD (p=0.006; p=0.0035 respectively),?-SMA (p=0.0029; p=0.0005 respectively) in response to TGF-ß was also inhibited. To discard and effect of the compounds on NOX1, PDGF (40 ng/mL) was used as ligand. Neither of the compounds altered the phosphorylation of ERK-1/2 or p38 (p>0.05). Conclusion: Our study establishes that acrylonitriles P1 and P3 inhibit ROS production through NOX4 inhibition, but not NOX1, in VSMC, affecting differentiation induced by TGF-ß.
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PT274PP

The intracellular pH is regulated by NHE1 and partially by V-ATPase or H+/K+-ATPase in HUVECs from gestational diabetes mellitus
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Introduction: Human umbilical vein endothelial cells (HUVECs) from GDM pregnancies showed alkaline intracellular pH (pHi). Na+/H+ exchanger 1 (NHE1), vacuolar ATPase (V-ATPase) or H+/K+-ATPase are involved in the pHi regulation. However, the involvement of NHEs, V-ATPase and H+/K+-ATPase in this phenomenon in cells from GDM is unknown. Objective: To determinate whether NHE, V- ATPase and H+/K+-ATPase are involved in pHi modulation in HUVECs from GDM. Methods: HUVECs were isolated (collagenase digestion) from full term normal (n = 11) or GDM (n = 8) pregnancies collected at the Clinical Hospital CHRISTUS-UC (Chile) (Ethics committee approval was obtained). The study conformed to the Declaration of Helsinki. HUVECs were cultured in medium 199 plus sera (20%) up to passage 2. The pHi was measured in cells loaded with the fluorescent pH-sensitive probe BCECF-AM (12 µmol/L, 10 min) and exposed to NH4Cl (20 mmol/L). Basal and pHi recovery rate (dpHi/dt) were estimated (up to 360 s) in cells exposed to 5 µmol/L 5-N,N-hexamethylene-amiloride (HMA, NHEs general inhibitor), 0.1 µmol/L zoniporide (Zn, NHE1 inhibitor), 0.1 µmol/L concanamycin A (V-ATPase inhibitor), or 10 µmol/L Schering (H+/K+-ATPase inhibitor). Results: HUVECs from GDM show higher (P<0.04, unpaired one-way ANOVA) basal pHi (7.75 ± 0.17) compared with normal pregnancies (7.12 ± 0.04) (values are mean ± SEM) in the absence of inhibitors. The dpHi/dt in GDM was higher (4.2 ± 0.2 fold) than in normal pregnancies, an effect reversed by Zn (90 ± 10%), concanamycin A (40 ± 4%), and Schering (45 ± 5%). However, the dpHi/dt in normal pregnancies was inhibited by Zn (58 ± 1%), concanamycin A (61 ± 19%), and Schering (14 ± 3%). Similar results were found when HMA was used. Conclusion: The pHi recovery requires NHE1, V-ATPases, and H+/K+-ATPase activity in HUVECs from women with GDM but mainly NHE1 and V-ATPases in cells from normal pregnancies.
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JG151TL

Human umbilical vein endothelial cells from gestational diabesity show increased intrinsic buffer capacity compared with cells from gestational diabetes
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Introduction: Gestational diabesity occurs in women with pre-gestational obesity that develops gestational diabetes mellitus (GDM). Human umbilical vein endothelial cells (HUVECs) from GDM pregnancies showed alkaline intracellular pH (pHi) and higher activity of Na+/H+ exchanger 1 (NHE1). The capacity of buffering changes in pHi, i.e. intracellular buffer capacity (βi) is unknown in HUVECs from gestational diabesity. Objective: To determine whether βi is altered in HUVECs from gestational diabesity. Methods: HUVECs were from full-term pregnancies from Clinical Hospital CHRISTUS-UC. Study groups: Normal pregnancies with

maternal pre-pregnancy normal weight (Nnw, n = 7), overweight (Now, n = 2) or obesity (Nob, n = 2), GDM with pre-pregnancy normal weight (GDMnw, n = 3), overweight (GDMow, n = 3), or obesity (GDMob or 'gestational diabesity', n = 3). The study conformed to the principles outlined in the Declaration of Helsinki. Basal and pHi recovery rate (dpHi/dt) were measured in cells loaded with BCECF-AM (12 µmol/L, 10 min). For βi determination, cells were exposed (3 min) to NH4Cl (50-0 mmol/L) in presence of 0.1 µmol/L concanamycin A (CoA, V-ATPases inhibitor), 10 µmol/L Schering (Sch, H+/K+-ATPase inhibitor) and in the absence of extracellular sodium. βi was calculated by the Henderson-Hasselbalch equation. Results: Basal pHi was increased (P<0.04, unpaired one-way ANOVA) in GDMnw (8.02 ± 0.07) and GDMow (7.73 ± 0.26) compared with Nnw (7.09 ± 0.04) or Now (7.13 ± 0.02) (mean ± S.E.M.). However, the pHi in GDMob (7.57 ± 0.10) was lower than GDMnw, higher than Nnw and Nob (7.13 ± 0.02). The βi at pHi 6.23 for GDMob was higher compared with all other groups except Now. Conclusion: HUVECs from gestational diabesity show better intrinsic buffering capacity than cells from GDMnw. This phenomenon is not enough to reach the buffering capacity seen in cells from normal pregnancies.
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JC644NJ

Angiotensin II type 2 receptor (AT2R) agonist, C21, prevents the epithelial cell damage caused by renal ischemia

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Introduction: During acute kidney injury induced by ischemia-reperfusion (IR), loss of cytoskeletal integrity and disruption of adherent junctions are rapid events in response to ATP depletion. Angiotensin II via AT2R participates in tissue repair. Our previous data in rats demonstrated that pretreatment with the AT2R agonist, C21, attenuated renal dysfunction caused by IR and induced better preservation of tubular architecture. RhoA and Cdc42 are Rho-GTPases involved in maintenance of renal tubule epithelial integrity. Objective: Evaluate the effects of C21 pretreatment on renal ischemia epithelial cell damage. Methodology: Male Wistar rats (n=6 per group) underwent 40 min unilateral renal ischemia + 1 day of reperfusion. C21, 0.3mg/Kg/d i.p., was administered for two days prior to IR. RhoA and Cdc42 protein abundance was evaluated in renal cortex by western blot. MDCK renal cells were grown on filters in conditions that assure well-defined epithelial polarity (n=3 per group). To simulate ischemia by ATP depletion, cells were exposed to antimycin A (10 uM) and 2-deoxyglucose (10 mM) during 90 min (I). Cells were pretreated with C21 1mM (I+C21) or vehicle (C+C21) during 24 h. Cells were analyzed by immunofluorescence using Faloidin and anti E-cadherin. Data are shown as mean±SEM. Statistics: ANOVA followed by Newman-Keuls test. Institutional Animal Care Committee approved this study. Results: IR downregulated cortical RhoA (-65%*) and Cdc42 (-55%*) abundance in rats. C21 prevented this decrease. In MDCK, C21 prevented the ischemia induced reduction of actin in brush border microvilli (Control (C): 44±2%; C+C21: 37±2; I: 21±2*; I+C21: 45±5) and in stress fibers (C: 28±2%; C+C21: 27±4; I: 6±1*; I+C21: 23±4). Membrane E-cadherin decreased in I (-13%*) and C21 prevented this change. *p<0.05 vs C. Conclusions: These results suggest a role of AT2R in the organization of the actin cytoskeleton and adherent junctions which could be mediated by Rho-GTPases.
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DN239TN

Consequences of aerobic training in myocardial mitochondria of hypertensive rats

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Introduction: Hypertensive myocardium is characterized by structural alterations and mitochondrial dysfunction. Aerobic training exerts beneficial cardiac adaptations to satisfy the work-load and improve its functionality. However, how it affects the cardiac mitochondria remains elusive. Objective: To evaluate whether swimming training promotes beneficial adaptations in myocardial mitochondria of spontaneously hypertensive rats (SHR).Methods. 3-month old SHR were randomized to sedentary (Sed) and trained (Ex) groups. After the swimming protocol (8 weeks, 5 days/week), the hearts were destined for transmission electron microscopy (TEM) imaging, RT-PCR analysis, citrate synthase activity determination, or mitochondrial isolation. Mitochondrial membrane potential and calcium content were measured in isolated mitochondria with Rhodamine-123 and Calcium Green-5N, respectively. Results are expressed as mean±SEM (n) (TEM results as median-IQR) and are statistically different by Welch or Mann-Whitney test (p<0.05). Otherwise, the p-value is stated. Protocols were approved by the Care and Use of Laboratory Animals Committee of our institution. Results: Aerobic training trended to restore the myocardial ultrastructural disarray present in Sed (clusters/photo, Ex: 4.63±1.07 (4), Sed: 8.45±0.84 (4), p=0.057) and increased mitochondrial morphological parameters: cross-sectional area (µm2, Ex: 0.79-0.74 (1266), Sed: 0.72-0.71(1518)) and aspect ratio (Ex: 1.90-1.13 (1266), Sed: 1.60-0.77 (1518)).

Also, improved the following mitochondrial function parameters: ΔΨm (mV, Ex: -175.2±5.8 (6), Sed: -148.6±9.2 (5)), mitochondrial calcium content (nmol/mg, Ex: 151.4±21.6 (6), Sed: 87.6±17.8 (7)), and citrate synthase activity (µmol/min*mg, Ex: 0.87±0.03 (5), Sed: 0.64±0.05 (5)). Training modified mitochondrial dynamics (% vs Sed (≥5)): mtDNA/nDNA: 154.0±21.6 (4) (p=0.082); PGC1-α: 149.4±19.0 (6); DRP-1: 309.4±77.5 (7); MFN1: 59.0±8.2 (7); PINK1: 209.9±49.4 (8) (p=0.063). Conclusion: These results support that aerobic training promotes the following favorable adaptations in cardiac mitochondria: 1) improved mitochondrial-sarcomere organization; 2) enhanced function: less depolarized ΔΨm, increased mitochondrial Ca+2 and citrate synthase activity; and 3) promoted dysfunctional mitochondrial clearance process: reduced fusion and enhanced biogenesis, fission, and mitophagy
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PF542RN

Subcellular discordant alternans in cardiac myocytes: characterization and susceptibility to pharmacological RyR2 modulation.
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Objectives: To characterize discordant calcium release in ventricular cardiomyocytes, and evaluate the impact of pharmacological inhibition of Ryanodine receptors (RyR2) on the occurrence and magnitude of discordance. Materials and methods: Ventricular cardiomyocytes were obtained from wistar rats (8-12 weeks old) after heart excision and enzymatic digestion. Cells were loaded with Fluo-4 and line-scanned in a confocal microscope to detect cytosolic Ca2+. Pharmacological inhibition of RyR2 was tested with 5 µM of both VK-II-86 and Dantrolene. Tree groups of cells (control, VK-II-86 and dantrolene) were field stimulated at 1 and 5 hertz. After 1 minute at each frequency electrical pacing was stopped to record spontaneous Ca2+ release (Ca2+ waves).Results: We found previously undescribed features of subcellular discordant alternans such as its reversibility after returning to basal pacing frequency and its instability promoted by the recruitment of out-of-phase Ca2+ release zones by its neighbors. A discordance index (DI) is proposed (Standard deviation of local alternans ratio) and correlated with the degree of negative staircase with a Pearson r of -0.66 (n= 15).The equipotent RyR2 inhibition obtained with VK-II-86 and dantrolene was evident when Ca2+ waves frequency was significantly reduced compared to control (ANOVA with tukey post-test). At both basal and high pacing frequency only VK-II-86 promoted a significant increase of DI (n=14-22 per group. ANOVA with tukey post-test).Conclusions: Subcellular discordant alternans are a reversible process induced by rapid pacing and can be characterized with a Discordance index which correlates with transient amplitude-frequency relationship and is sensitive to pharmacological RyR2 modulation. Interestingly, differential response to VK-II-86 and dantrolene was observed, suggesting that RyR2 modulation and prevention of Ca2+ waves occurrence can be reached with normal or high chance of promoting discordant Ca2+ release.
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GD615QQ

PDGF-C reduces mitochondrial damage induced by high D-glucose in Human Aortic Endothelial Cells
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Introduction: Hyperglycemia in diabetic patients is a risk factor for the development of cardiovascular diseases (CVDs). Loss of endothelial cells (ECs) function is a marker for CVDs and it is mainly related to increase production of oxygen reactive species (ROS). Vascular Endothelial Growth Factor (VEGF) is essential in stimulating endothelial function and mitigating ROS impact; however, VEGF signaling is partially inhibited in diabetes. Therefore, looking for factors that preserve/improve the EC function in high D-glucose (HG) is an interesting field of research. Platelet Derived Growth Factor C (PDGF-C) is a peptide that stimulates angiogenesis and revascularization in ischemic tissues of diabetic mouse and promotes the migration of progenitors and mature ECs to injury sites; however, the molecular mechanisms of its actions have not been described. Objective: To evaluate the effect of PDGF-C on ECs damage induced by HG. Methods: Human Aortic Endothelial Cells (HAECs) were grown in glucose concentrations ranging from 5mM (NG) to 35mM (HG) for 1 to 48 h. Treatment with 50 ng/ml PDGF-C was made for 1 to 3 h. Cytosolic and mitochondrial ROS were measured by fluorometry, expression of antioxidant enzymes and mitochondrial dynamics proteins were evaluated by Western blot, integrity of mitochondrial network was measured by confocal microscopy. Data are expressed as mean ±SEM, one-way ANOVA was used for comparisons between groups. Results: HG for 6 h induced mitochondrial, but not cytosolic ROS ($p<0.001$). PDGF-C reduced the increase of mitochondrial ROS induced by HG ($p<0.001$) and up-regulated the expression of Catalase and SOD2, but no GPx1 (n=2). HG reduced the count and length of branches, junctions and total area of the mitochondrial network (n=6). PDGF-C restored the integrity of mitochondrial network fragmented by HG (n=6) and up-regulated the expression of mitochondrial fusion proteins. Conclusion: PDGF-C modulated the mitochondrial damage induced by HG level in HAECs.

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TS943HR

Effect of Hsp60 Peptides on Cell Homeostasis

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Introduction: Heart failure (HF) is a chronic disease characterized by the inability of the heart to pump enough blood into the systemic circulation to meet the metabolic demands of peripheral tissues. It is estimated that only in Mexico at least 2 million people are affected by this disease, which poses a great epidemiologic problem nationwide, while also being an important cause of mortality worldwide. Heat shock proteins (HSP) play an important role in cardiovascular disease because of their function as modulators of the innate and adaptive immune responses. In patients with HF, HSP60 have an abnormal distribution within the cell and an increased apoptotic activity and activation of the innate immune response, events that further promote a systemic proinflammatory state that perpetuates the progression of HF. Moreover, some peptides derived from HSP60 may induce inflammatory responses after being recognized by the immune system (Krishnan-Sivadoss, 2020), however, the effect that these peptides may have on other cell types, particularly non-immune cells such as cardiomyocytes, which also express PRRs, is still unknown. Objective: To determine the effect of three selected HSP60 peptides in cardiomyocytes and endothelial cells in order to study their possible role in the development of the cardiovascular disease. Methods: H9c2 and C166 cells were incubated for 24h in DMEM/1% FBS to reduce interference that growth factors present in serum could have on lipopolysaccharide and peptide-induced toxicity cells. Determination of LD50 for LPS and HSP60 peptides were performed with Alamar Blue. Western Blot measured peptide-induced changes in cell protein expression. Data were analyzed by ANOVA followed by Dunnett's multiple comparisons tests using GraphPadInStat. Results and conclusions: Peptides induced different cell viability effects and changes in protein expression. Treatment with some HSP60 peptides induced a 46% viability reduction and increases in protein expression for TLR4 and IL6, 130% and 240%, respectively.

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MM886DF

PLGA loaded resveratrol PLGA nanoparticles improve protection response in H9c2 cells against hypoxia-reoxygenation damage

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Introduction: Increased incidence of cardiovascular diseases has become an important public health issue worldwide, promoting the development of drug delivery strategies for compounds suitable as coadjutant therapies. Resveratrol (RSV) is a polyphenolic compound that has shown cardioprotective activity mainly due to SIRT1/3 stimulation, leading among other effects to a reduction of ROS [1], a key factor in many cardiovascular diseases, such as Ischemia-Reperfusion injury and hypertrophy. However, due to its structural and biological properties, RSV presents poor solubility, low stability, high metabolization rate and low bioavailability, which are the most important challenges to improve its therapeutic effects in vivo [2]. Objective: To develop a nanovector that encapsulates RSV and study its nanobiointeractions and potential cardioprotection in an in vitro cardiac model. Methodology: RSV was encapsulated in poly(lactic-co-glycolic acid) (PLGA) by evaporation method of oil-in-water emulsion [3] to evaluate the cardioprotection of nanoencapsulated RSV against hypoxia-reoxygenation damage in H9c2 cells. Results were obtained from independent (n=3) measurements and presented as mean ± SEM (standard error of the mean), considering a P value <0.05 for statistical significance. Results and conclusion. We designed a negatively charged PLGA-RSV nanoparticle (-13.3 ± 0.97 mV) with monodisperse size distribution (PDI=0.214), and an average hydrodynamic diameter of 145.97 ± 0.97 nm. The physicochemical characterization of the nanosized formulation resulted in 15.83% of RSV loading, and the detection of the characteristic absorption bands for the PLGA-RSV formulation through FTIR analysis (1750, 1100 and 1000 cm-1 for PLGA and 1590 and 3250 cm-1 for RSV). Then, we evaluated cardioprotection of the nanostructured RSV in relation to free RSV under conditions of hypoxia-reoxygenation damage in H9c2 cells. Finally, we determined the association of nanoparticles with H9c2 cells using PLGA-RSV nanoparticles labeled with FITC.[1] DOI: 10.1155/2017/1750306[2] DOI: 10.1016/j.jconrel.2011.09.083[3] DOI: 10.1155/2019/7683051

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KQ685NL

Angiotensin-(1-9) prevents lipotoxic stress-induced hypertrophy in cultured cardiomyocytes

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Introduction: The classic renin-angiotensin system (RAS) has a counter-regulatory axis known as alternative RAS with cardioprotective action on the properties of cardiomyocytes. Angiotensin-(1-9) (Ang-(1-9)) blocked mitochondrial fission in a model of norepinephrine-induced cardiomyocyte hypertrophy. This latter cellular process is characterized by increases in the size of cardiomyocytes, the number of sarcomeres, the content of contractile proteins (i.e., the beta-myosin heavy chain), and by the re-expression of the fetal gene program (i.e. ANF). Moreover, mitochondria are fragmented in this process, thus decreasing oxidative metabolism. Interestingly, excessive accumulation of lipids (lipotoxicity) in the heart also promotes morphological and metabolic changes in cardiomyocytes, including the development of cardiac hypertrophy. Aim: To evaluate if Ang-(1-9) prevents the effects of lipotoxicity on cardiac hypertrophy. Methodology: Neonatal rat ventricular myocytes (NRVM) were isolated from newborn (2-3 days) Sprague-Dawley rat hearts in a procedure approved by the institutional committee for the care and use of animals of the University of Chile. NRVMs were treated with or without 100 µM Ang-(1-9) for 6 h before treatment with palmitate (328 µM for 24 h) and then, hypertrophic changes were evaluated. Different hypertrophic markers were assessed, including β-MHC and ANP protein levels by Western blot. Cell area, perimeter, and mitochondria mean volume and number were analyzed by confocal microscopy. Results are expressed as mean ± SEM. Statistical analysis corresponds to the T-test or one-way Anova analyses of 2-4 independent experiments (N). Results: Palmitate (328 µM 24 h) significantly increases the levels of β-MHC and ANP, increases the area and perimeter of the NVRMs and triggered mitochondrial fragmentation. Ang-(1-9) prevents the increase of β-MHC, suggesting that it could prevent cardiomyocyte hypertrophy and the changes in mitochondrial morphology induced by lipotoxicity. Conclusions: Palmitate promotes cardiomyocytes hypertrophy together with changes in mitochondrial morphology. Ang-(1-9) prevents lipotoxicity-induced cardiomyocyte hypertrophy.
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KD851GG

Neuronal activity modulates pericyte interactions via pannexons: characterization, mechanisms and implications

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Introduction: To avoid energy deprivation, the cerebral local blood flow (BF) increases in active areas to adjust glucose and oxygen supply to neurometabolic demands, a response known as functional hyperemia. The Neurovascular Unit coordinates this process; however, its intimate mechanisms and regulation have not been resolved. In the neuro-microvascular interface, the contractile pericapillary pericytes, sense the neuronal activity and undergo relaxation increasing the BF. Brain pericytes express large-pore channels formed by pannexin1 (pannexons); open pannexons release ATP, increasing intracellular calcium. Objectives: Characterize the role and regulation of Pannexin1 in cerebral pericapillary pericytes during increased neuro-metabolic demands. Methods: Using pharmacological tools, functional imaging and molecular biology, we evaluated pannexons activity in hippocamapal pericytes of wild-type and pannexin1 knock-out (Panx1-/-) mice in resting conditions and upon increased neuronal discharge, in both ex vivo and in vivo conditions. We employed Mann-Whitney and Kruskal-Wallis tests for statistical analysis. The Animal Experimentation Committee of Universidad de la República, Montevideo-Uruguay, approved all animal procedures. Results: Epileptic seizures (in vivo) induced by picrotoxin-PTX (1mg/kg; 8mg/kg ip), a GABA-A receptor blocker, significantly inhibited hippocampal pericyte membrane permeability as compared to control (vehicle). This effect was confirmed ex vivo in PTX-treated (100µM; 45min) acute hippocampal slices. Genetic ablation of pannexin1 (Panx1-/- mice), or administration of tetrodotoxin (TTX; 0.5µM), an inhibitor of voltage-gated sodium channels or DPCPX (5µM; 10µM), an A1 adenosine receptor antagonist, but not MRS1754 and ANR-94, respectively A2a and A2b adenosine receptor antagonists, totally prevented PTX-inhibition. Adenosine mimicked PTX effects in slices and cultured pericytes. Conclusion: Our results indicate that neuronal activity decreases the molecular exchange between brain pericytes and their microenvironment under both ex vivo (slices) and in vivo (awake animal) conditions; this effect is due to the closure of pannexons and could be mediated by adenosine through A1 receptors, probably via a direct effect.
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Mitochondrial NHE1 and NCLX in the diabetic cardiomyopathy

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Introduction: Heart failure is the leading cause of death among diabetic people. Cellular and molecular entities leading to diabetic cardiomyopathy (DCM) are poorly understood. Na+/H+ exchanger (NHE) is a ubiquitous ion exchanger responsible for intracellular

pH maintenance. NHE1 is the heart isoform. Our previous results show increased NHE1 activity in the heart from obese and diabetic mice (ob-/-) compared to the control heterozygous littermates (ob+/-). Mitochondrial dysfunction has been related to the development of heart failure and NHE1 has been detected in rat mitochondria, where its inhibition resulted in decreased mitochondrial swelling. Altered mitochondrial Ca2+ may result in mitochondrial dysfunction. The role of mitochondrial NHE1 and Na+/Ca2+ exchanger (NLCX) in DCM has not been yet studied. Objective to study the role of NHE1 and NLCX in the mitochondria of ob-/- mice. Methodology: Left ventricle mitochondria were isolated by differential centrifugation. NHE1 inhibition was obtained using 10 µM HOE. Data were expressed as mean ± SE. Statistical analysis was performed by one-way ANOVA followed by Tukey's test. The experimental protocol was approved by the Animal Welfare Committee of La Plata School of Medicine. Results: Our results showed increased NHE1 expression in the mitochondria from ob-/- mice. Preliminary results also show increased NCLX expression. These mitochondria also presented altered swelling, mPTP opening, CRC, and ΔΨm, while NHE1 blockade partially reverted this phenotype. Finally, mitochondria from ob-/- mice present reduced calcium content and increased NCLX expression. Conclusions: The role of mitochondrial NHE1 is not completely understood however, NHE1 activity could not directly result in ΔΨm alteration considering its electroneutral exchange. However, alterations in mitochondrial Na+ concentration could influence NCLX activity. Considering that NHE1 inhibition partially reverses mitochondrial alterations and that our preliminary data indicates increased NCLX, our results indicate that both mitochondrial NHE1 and NCLX are involved in the development of mitochondrial dysfunction in DCM.
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FD814JL

Maternal DHA supplementation reverts the higher expression of proinflammatory genes and DNA methylation patterns in the offspring´s cord blood monocytes of women with Pregestational Obesity.

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The offspring of women with pregestational obesity (PGO) have an impaired innate immune function. Docosahexaenoic acid (DHA) supplementation during pregnancy decreases maternal systemic inflammation. Its effects on fetal immune response remain unknown. Aim: To determine the effect of DHA supplementation during pregnancy on gene expression, function, and DNA methylation of PGO neonatal monocytes. Methods: Pregnant women with PGO (BMI ≥30 kg/m2) participating in the RCT NCT02574767, were supplemented with DHA: 200 mg/day (PGO-200, n=18) and 800 mg/day (PGO-800, n=21). Control group was normal-weight women (NW, n=20). At delivery, cord blood monocytes (CMo) were isolated to determine pro-inflammatory (MCP1, TNFα, IL-6, IL-8) and anti-inflammatory (PPARγ, PGC1α, IL-10) mRNA by RT-qPCR, and methylome analysis (EPIC-850K, Illumina®). NW and PGO CMo (n=8) were stimulated in vitro with Lipopolysaccharide (LPS), DHA, and DHA receptor antagonist, and then mRNA and protein levels were measured by RT-qPCR and CBA BD Biosciences, respectively. All subjects provided signed consent. Study approved by the Institutional Ethics Review Board of PUC. Statistical: Kruskal-Wallis test, STATA. Results: PGO-200 CMo expressed higher mRNA levels of MCP1, TNFα, IL-6, IL-8, PGC1α, and IL-10, but lower mRNA levels of PPARγ compared to NW. Pro-inflammatory changes and PPARγ levels were reverted in PGO-800 nearly to NW values. Hypermethylated sites were found in PGO-800 compared to NW, mainly in the gene body and intergenic regions of genes related to immune response, and metabolism. In vitro LPS stimulation induced CMo expression of MCP1, IL-6, and IL-10, in both groups and it was reversed by DHA. However, PGO-CMo had a blunted response to LPS challenge compared to NW. These effects were independent of the DHA receptor (GPR120) activation. Conclusion: PGO induces the expression of inflammatory genes in CMo, with a blunted response to LPS. Maternal DHA supplementation reverts these changes and modulates DNA methylation in a DHA receptor-independent manner.
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CB817BM

Study of the biochemical and behavioral effects of omega-3 fatty acids on normotensive and hypertensive rats

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Introduction: The dietary intake of polyunsaturated fatty acids (PUFAs) of the omega-3 series -eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)- reduces the risk of cardiovascular diseases (CVD) and produces beneficial effects in patients with

neurodegenerative diseases. The incorporation of omega-3 PUFAs into biological membranes changes membrane properties, and thereby affect signal transduction and cell function. Objectives: Analyze the effect of early supplementing in diet with EPA and DHA over depression symptoms and cardiac function in spontaneous hypertensive rats (SHR) compared with Wistar (W). Materials and Methods: Twenty one-day-old male Wistar and SHR rats (N=25) were randomly separated into four groups: Wistar, control and treated (W; W-T), and SHR, control and treated (SHR; SHR-T). The systolic pressure (SP) was measured and the Marble anxiety test was performed the day before the sacrifice. Blood, cardiomyocytes, and hippocampus were isolated and the total free fatty acids (FFA) composition was analyzed. Statistical analysis was performed by One-way ANOVA, followed by Tukey's multiple comparisons test, p<0,05. Handling and sacrifice of the animals were in full accordance with the Committee for the care and management of laboratory animals, UNLP (CICUAL). Results: Lower omega-6/omega-3 indexes were detected in the plasma of both W-T and SHR-T animals. Strikingly, the DHA percentage and DHA/Arachidonic Acid ratio were significantly higher in SHR than in W cardiomyocytes. No significant effect on the SP of SHR-T animals was detected. Similar levels of omega-3 PUFAs were found in the hippocampus of both W and SHR animals. Notwithstanding, a significant reduction in cardiac hypertrophy parameters and an increase in the digging activity of SHR-T animals were registered. Conclusion: These preliminary results show that omega-3 PUFAs intake could contribute to the prevention of the chronic inflammation associated with CVD and neurodegenerative diseases. However, the omega-3 PUFAs enrichment of biological membranes may not be the underlying mechanism responsible for these omega-3 FAs' beneficial effects.

TS325KN

Effect of glucose and insulin on cell proliferation of MCF7 cells measured by Incucyte

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Introduction: In recent years, a close relationship has been observed between insulin resistance, type 2 diabetes mellitus and cancer. In fact, many malignant neoplasms, including mammary carcinoma, report an increase in the relationship between expression of insulin receptor isoform A and B, a phenomenon linked to an increase in cell proliferation via RAS / RAF / MEK / ERK. However, the molecular mechanism by which insulin is associated with cancer is still unknown. Aim: To determine cell proliferation under conditions of insulin resistance in MCF7 cells. Methodology: Cell cultures of MCF7 were incubated with DMEM under conditions of high glucose (25 nM) and normoglycemia (5 mM) in the presence and absence of insulin (0 - 100 nM for 24 hours). The proliferative behavior of the cells was monitored by means of the Incucyte for 24 hours. Statistical analysis considered a Mann-Whitney post-analysis and a value of p <0.05 as statistically significant, using the GraphPad Prism7 software. Results: In absence of insulin, MCF7 cells proliferated more in the normoglycemic condition than in the high glucose condition. Interestingly, insulin was able to reverse the reduction in proliferation induced by high glucose. The high glucose condition was more effective in stimulating cell proliferation, especially at concentrations of 0.01 nM and 0.1 nM with respect to cells without insulin stimulation. Conclusions: High glucose reduced cell proliferation in MCF7 cells cultured in the absence of insulin. Also, insulin was able to reverse the reduction in the proliferation of MCF7 cells generated by high glucose, and the greater proliferative effect induced by insulin was observed at physiological and non-supraphysiological concentrations.

MT988MJ

Effect of glucose and insulin on cell proliferation of MCF7 cells measured by Incucyte

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Introduction: In recent years, a close relationship has been observed between insulin resistance, type 2 diabetes mellitus and cancer. In fact, many malignant neoplasms, including mammary carcinoma, report an increase in the relationship between expression of insulin receptor isoform A and B, a phenomenon linked to an increase in cell proliferation via RAS / RAF / MEK / ERK. However, the molecular mechanism by which insulin is associated with cancer is still unknown. Aim: To determine cell proliferation under conditions of insulin resistance in MCF7 cells. Methodology: Cell cultures of MCF7 were incubated with DMEM under conditions of high glucose (25 nM) and normoglycemia (5 mM) in the presence and absence of insulin (0 - 100 nM for 24 hours). The proliferative behavior of the cells was monitored by means of the Incucyte for 24 hours. Statistical analysis considered a Mann-Whitney post-analysis and a value of p <0.05 as statistically significant, using the GraphPad Prism7 software. Results: In absence of insulin, MCF7 cells proliferated more in the normoglycemic condition than in the high glucose condition. Interestingly, insulin was able to reverse the reduction in proliferation induced by high glucose. The high glucose condition was more effective in stimulating cell proliferation, especially at concentrations of 0.01 nM and 0.1 nM with respect to cells without insulin stimulation. Conclusions: High glucose reduced cell proliferation in MCF7 cells cultured in the absence of insulin. Also, insulin was able to reverse the reduction in the proliferation of MCF7 cells generated by high glucose, and the greater proliferative effect induced by insulin was observed at physiological and non-supraphysiological concentrations. Funding: FONDECYT 11170710
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LB868DS

Role of TLR4 in the inflammatory response observed during renal ischemia and reperfusion

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Introduction: Renal ischemia and reperfusion (I/R) cause acute kidney damage and activates Toll-like membrane receptors (TLRs). The TLR4 receptor plays a critical role in inflammation and reparation during I/R. Therefore, deciphering the molecular mechanisms associated with the role of TLR4 during I/R damage is necessary. Objective: To determine the role of TLR4 during renal I/R in factors that induces or prevent the inflammation using Wild type (Wt) and Knockout (KO) animals for TLR4. Methodology: Mice (2-3 months of age) C57BL/6 Wild type (Wt) and TLR4 Knockout (KO) were subjected to a model of renal I/R, approved by the Bioethics Committee of the Universidad de Los Andes. The I/R (30 min/48 hours). Sham controls were used. Cytokines IL-1β, IL-6, IL-10, and IL22 (rRT-PCR) and NGAL as kidney damage markers (Western blot) were measured. Furthermore, morphological alterations were evaluated through Hematoxylin and Eosin stains and Periodic Acid Schiff (PAS). Data were expressed as mean + SEM. Wt and KO (n=4). Mann Whitney test (U-test), * $p<0,05$. Results: The results with H/E and PAS staining showed that Wt and KO showed tubular damage induced by I/R. NGAL was increased by I/R in the cortex and medulla of Wt and KO animals. The mRNA of IL-1b did not change in the studied groups. The IL-6 was significantly upregulated during I/R in the cortex of Wt and KO. However, IL-10 was only induced in the cortex of Wt animals by I/R, but not in KO. In the medulla, no differences were observed. The IL-22 mRNA levels were not detected in kidney samples studied. Conclusions: The absence of TLR4 during the I/R renal did not significantly prevent kidney injury (NGAL), tubular damage, and IL-6, but the induction of IL-10 by I/R was not induced when TRL4 was not expressed, suggesting that the reparation process was impaired.

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LN816TP

Pro-fibrotic activity induced by Deoxycholic and Cholic acids in skeletal muscle cells

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Introduction: Fibrosis is a condition that can participate in muscle weakness; this is characterized by an increase in the extracellular matrix (ECM) components such as fibronectin. This affection in the skeletal muscle has been associated with chronic diseases. Chronic Liver Disease (CLD) is a condition in which there is muscle weakness. A hallmark of this disease is bile acids (BA) increase in the blood, such as cholic acid (CA) and deoxycholic acid (DCA). Skeletal muscle presents the TGR5 membrane receptor for BA. Patients with muscle weakness due to CLD have a decrease in muscle mass However, the presence of muscle fibrosis linked to this pathology has not been evaluated. Aim: In this work, we evaluated whether BAs could increase the fibrosis marker fibronectin protein levels in cells and if activation of TGR5 participates in this effect. Methodology. Fibroblast, myoblast, and myotubes were incubated with DCA (n=3) and CA (n=3). Also, we used a specific agonist for TGR5 (INT-777) (n=3 except myoblast). One-way ANOVA was used for statistical analysis, considered statistically significant $p<0.05$. Approved by Bioethics Committee Universidad Andrés Bello (number: 007/2016).Results: We observed a differential effect depending on the cell population. In fibroblasts, DCA and CA increase fibronectin protein levels. The same response was found when activating TGR5 with INT-777. Meanwhile, in myoblasts and myotubes, DCA reduced fibronectin. In contrast, CA not produce any effect in this fibrotic marker. Similarly, INT-777 not induce changes in fibronectin levels in myoblasts and myotubes. Conclusion: These results indicate that DCA and CA have a fibrotic effect on fibroblasts, as well as the activation TGR5 by INT-777. On the other hand, both myoblasts and myotubes suggest an antifibrotic effect when treated with DCA.

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DG488SM

Fish oils modulate endoplasmic reticulum stress and autophagy in soleus muscle disuse induced atrophy in rats

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Introduction: The effects of omega-3 supplementation on endoplasmic reticulum (ER) stress and autophagy signaling pathways in disuse muscle atrophy are unknown. Aim: To investigate the effects of high EPA or high DHA fish oils on soleus muscle ER stress and autophagy signaling pathways in hindlimb suspension (HS) - animal model. Methodology: Adult male rats (n=60) received daily oral supplementation (0.3 mL/100 g b.w.) of mineral oil (MO) or fish oils for two weeks. Afterward, we subjected half the rats (n=30, 10 rats with each supplementation: MO, high EPA or high DHA) to HS and the respective treatments concomitantly for a further two-week period. At the end of four weeks, we evaluated: body weight, soleus muscle mass, composition of fatty acids in the gastrocnemius muscle, and ER stress (p-IRE1, splicing of XBP1, JNK1/2, p38, BiP, PDI, CHOP, caspase 3, and PERK) and autophagy (BECLIN, LC3 II, and ATG14) signaling pathways in the soleus muscle. We analyzed the results by two-way ANOVA (with Bonferroni posthoc test) and presented as mean ± SEM. Ethics Committee of the Institute of Biomedical Sciences, University of Sao Paulo, approved this study. Results: HS decreased body weight gain, soleus muscle mass, and altered ER stress and autophagy signaling pathways in the soleus muscle. Both fish oils decreased the ratio of omega-6/omega-3 fatty acids in the gastrocnemius muscle. EPA-rich fish oil decreased BECLIN expression and attenuated the increase of ATG14 expression induced by HS. DHA-rich fish oil increased p-IRE1 content in HS rats and decreased BiP expression in the control condition. Both fish oils attenuated the decrease of BiP content and BECLIN expression and the increase of IRE1 and PERK expression induced by HS. Conclusion: Both fish oils (high EPA and high DHA) modulated ER stress and autophagy signaling pathways in soleus muscle HS induced atrophy.
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FB983QT

Nerve-dependent activity sensing by MEF3 regulatory binding sites: a potential role of the nerve in controlling the cellular localization of SIX and EYA proteins

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Introduction: SIX1/4 homeoproteins participate as master regulators of myogenesis, controlling the fiber type diversity in the primary myotome. SIX proteins act synergistically with EYA proteins, by promoting their nuclear localization and the activation of MEF3 regulatory binding sites in the promoter regions of fast/glycolytic muscle genes. In slow/oxidative adult muscle fibers, the forced expression of SIX1 and EYA1 produces a reprogramming from a slow to a fast phenotype. The adult muscle phenotype is controlled by the pattern of electrical activity imposed from the α-motoneurons to the muscle fibers. However, it is unknown whether disrupting the normal signals from the nerve could modify the transcriptional activity of MEF3 sites. Objectives: The aim of this work was to evaluate the role of the nerve in controlling the transcriptional activity of MEF3 regulatory binding sites. Methodology: Five muscle groups of the lower limbs of BALB/c mice were electroporated with plasmids coding for a 6XMEF3-luc reporter. After 7 days, a group of animals was denervated and then, 14 days after electroporation, the luciferase activity was assessed in the muscles of control and denervated animals. Statistical analysis was performed using one-way ANOVA, followed by Tukey's multiple comparisons test. The data are shown as means ± SD, n = 4. All experiments were performed under the approval of the Bioethical Committee of the University of Chile. Results: In innervated conditions, the 6XMEF3-luc activity was 47-70% higher in fast- than in slow-type muscles. However, denervation caused a 51-60% reduction in 6XMEF3-luc activity in fast-type and a 50% increase in slow-type muscles ($P \leq 0.05$).Conclusion: In conclusion, we showed that MEF3 activity is altered in a nerve- and a phenotypic-dependent manner in both fast- and slow-type muscles.

KF767FQ

The effect of different antirheumatic agents: on the risk of insulin resistance and type 2 diabetes mellitus. Systematic review.

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Introduction. The immunosuppressive therapy required by a patient with rheumatoid arthritis (RA) is a risk factor for type II diabetes mellitus (DM2) and can increase the risk of getting an infection. It was compared the use of glucocorticoids (GC), classic and biological disease-modifying antirheumatic drugs (DMARDs), and Janus kinase Inhibitors (JAKi), in the risk of insulin resistance (IR) and DM2.General objective.●To compare the use of GC, DMARDs, and JAKi in the risk of IR and DM2.Specific objectives.● To correlate the use of GC and DMARDs with age, sex, obesity, and infections.●To identify drugs between GC and DMARDs that trigger IR in patients with RA.●To recognize DMARDs and JAKi type drugs that improving glycemic control in patients with RA.Methodology. A descriptive systematic-review, 5-year period. There were included 83 articles with contributions so much about RA as DM, GC and DMARDs. Keywords: RA, DM, GC, DMARDs, individually or by combination. PUBMED, SPRINGER, GOOGLE SCHOLAR, SCIENCE DIRECT. Results. Use of GC and DMARDs predisposes to infections, favored by increasing age and female sex; in obesity, there is a contradictory effect due to the expression of the GC receptor in adipocytes. Its use increases the risk of DM at doses of ≥7.5 mg daily. With respect to DMARDs; Anakinra significantly reduced glycosylated hemoglobin by inhibiting IL-1. TNFi such as Infliximab, Etanercept, and Adalimumab, reduces the risk of developing DM2 by 51% . They also improve IR in patients

with RA, as does Hydroxychloroquine. Tofacitinib and aspirin at low-dose inhibit the JAK-STAT and Nuclear factor-κB (NF-κB) signaling systems, this is a viable option to improve IR .Conclusion (s). GC produce adverse effects at high doses. The classic and biological DMARDs individually or collectively decrease DM2 and the JAKi could decrease, the likelihood to develop IR and hyperglycemia in DM2, in patients with RA.
Acknowledgments: We thank Dr. Emilio Bermudez (Universidad Metropolitana, Colombia) for his contribution to the search for bibliographic sources.

QH593RQ

Human trophoblast invasion and endothelial-like differentiation is regulated by O2 and TGF-ß1 through fibronectin expression/deposition: hypothesis of its role in preeclampsia

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Introduction: A satisfactory pregnancy outcome relies on adequate placental development. This depend on the invasion of the maternal decidua and subsequent remodeling of the spiral arteries by the fetal trophoblast. If these processes are not carried out successfully, serious pregnancy problems such as preeclampsia will develop. Oxygen (O2) tension and transforming growth factor type β1 (TGF-β1) are among the most important regulators of trophoblast invasion and differentiation. Increased placental levels of TGF-β1and low levels of O2 tension (hypoxia) are common features of preeclampsia. Fibronectin (FN) is an extracellular matrix (ECM) constituent, regulating cellular signaling, cytoskeletal rearrangements, invasion and differentiation processes. FN expression is stimulated by TGF-β in several human cells and its assembly in the ECM is orchestrated by O2 tension. However, whether FN modulates trophoblast invasion and endothelial-like differentiation in response to TGF-β1 and O2 tension has not been described. Aim: Determine the effect of O2-tension on FN expression in response to TGF-β1 and its role on human trophoblast invasion and endothelial-like differentiation. Methods: Human trophoblast HTR8/SVneo were treated with TGF-β1 under different O2-tension. FN expression was evaluated by Western Blot and qPCR. Transwell invasion assays were performed. Endothelial-like transition was assessed by Endothelial tube formation assay and evidenced by ImageJ software analysis and expression of differentiation markers. Project has the approval of ethics committee Universidad San Sebastian, period 2018-2022.Results: TGF-β1 induces the expression of FN trough the canonical and non-canonical pathways. Low O2-tension reduced the expression of FN and the TGF-β1-dependent FN expression. Trophoblast invasion is reduced while the endothelial-like differentiation is stimulated by high O2-tension and TGF-β1. Statistical comparisons were analyzed using ANOVA and Bonferroni's multiple comparisons test. Data are expressed as mean SD, n=3 for each experiment. = $P<0.05$. Conclusion: TGF-β1 and oxygen tension regulate trophoblast invasion and endothelial-like differentiation in a FN-dependent way.
FONDECYT 1180935 to Jaime Gutiérrez (Universidad San Sebastián, Chile). CONICYT, PAI Convocatoria Nacional Subvención a Instalación en la Academia, Convocatoria Año 2019, Folio N°PAI77190060.

CF463NT

Cardiac MUL-1: a novel RIDD substrate in palmitate-induced lipotoxicity?

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Introduction: The endonuclease activity of IRE1-alpha is the critical regulator of Xbp1 unconventional splicing and also responsible for IRE1-dependent decay of mRNA (RIDD), affecting several mRNAs. Decreased cardiac IRE1-alpha endonuclease activity caused by its nitrosylation has been found to be required during the development of heart failure with preserved ejection fraction (HFpEF) triggered by using a high-fat diet and L-NAME. MKC8866 is a selective IRE1-alpha endonuclease activity inhibitor that allows to study the levels of several RIDD targets. MUL1 is a mitochondrial E3 ubiquitin-protein ligase-1 induced by lipotoxic stress and participates in the degradation of Akt and some mitochondrial proteins. However, it remains unknown how cardiac MUL-1 protein levels are regulated. Aim: To investigate whether MUL-1 levels are regulated by RIDD under lipotoxic stress. Methods: Cultured rat cardiomyocytes were treated with BSA-palmitate containing media or control BSA-media, in the presence or absence of IRE1-alpha endonuclease inhibitor MKC8866. Protein levels of MUL-1, total IRE1-alpha, phospho-IRE1-alpha, and tubulin were assessed by Western blot. Results were mean ± SD (n=3) and analyzed by one-way ANOVA. The project complies with bioethics (20352-CYQ-UCH). Results: Palmitate-induced lipotoxicity triggers a chronic IRE1-alpha activation, which could lead to decreased levels of some RIDD targets. Turning off the endonuclease activity of IRE1-alpha with MKC8866, we observed increased protein levels of IRE1-alpha (known RIDD target) and MUL-1 (novel RIDD target). Conclusions: MUL-1 could be a novel RIDD target because its levels are increased in the context of decreased IRE1-alpha endonuclease activity associated with lipotoxicity
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HL775JK

Role of the A2B adenosine receptor in the generation of angiotensin 1-8, 2-8 and 2-7 in a model of diabetic nephropathy.

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Introduction: The progression of renal fibrosis in diabetic nephropathy (DN) has been linked to the local activation of the renin angiotensin system (RAS). However, the effect of some RAS peptides generated by aminopeptidase A (AP-A) and angiotensin converting enzyme 2 (ACE2) such as Ang 2-7 and 2-8 remain unclear. High plasma adenosine levels have been observed in DN patients and in experimental models of renal fibrosis. Consequently, in vivo treatment with MRS1754, an A2B adenosine receptor (A2BAR) antagonist, reduces glomerular fibrosis in diabetic rats. Objective: We aim to elucidate if the reduction of glomerular fibrosis by the antagonism of A2BAR is related to changes in AP-A, ACE2, and Ang 1-8, Ang 2-7 and 2-8 levels. Methodology: Streptozotocin-induced diabetic rats (DM) were treated with the A2BAR antagonist MRS1754 (DM+MRS1754) for 8 weeks. Physiological parameters were measured weekly. After finishing the treatment blood samples were taken and renal glomeruli were isolated. Serum and glomerular Ang 1-8, 2-7 and 2-8 levels were measured by ELISA; AP-A and ACE2 glomerular expression was evaluated by western blot and immunofluorescence. Animal procedures were approved by the Institutional Committee on the Use of Live Animals in Research. Statistically significant differences were determined by Student's t-test, values are means ± standard deviation. n=4. Results: MRS1754 treatment decreases proteinuria and glomerulosclerosis. Glomerular AP-A and ACE2 expression decreased in DM, while MRS1754 reversed it. Both Ang 1-8 and 2-8 were augmented in serum and in the medium of purified glomeruli from DM rats; differently, serum Ang 2-7 declined. Serum Ang 1-8 and 2-8 levels diminished in DM+MRS1754 rats whereas Ang 2-7 increased in serum and medium of glomeruli from MRS1754-treated rats. Conclusion: MRS1754 treatment in DN rats increases the glomerular expression of AP-A and ACE2, reversing diabetic activation of RAS and producing an improvement in renal functionality.

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BP322HS

Effect of moderate exercise on skeletal muscle mitochondrial function and dynamics in obese Zucker rats.

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Introduction: Obesity and metabolic syndrome are related to a significant reduction in mitochondrial quality. Evidence suggests that both conditions lead to unbalanced fusion and fission, the main events of mitochondrial dynamics and this is associated with alterations in mitochondrial function, mainly in highly energetic tissues such as skeletal muscle. In the treatment of metabolic disorders, the various adaptations resulting from moderate-intensity exercise stand out, considered to be regulated by AMPK. Objectives: This project aimed to evaluate modifications in expression of the main regulators of mitochondrial dynamics and AMPK content and activity; to determine changes in mitochondrial function through evaluation of membrane potential and mitochondrial distribution in the mitochondrial subpopulations using confocal microscopy and to evaluate mitochondrial biogenesis in skeletal muscle using a murine model of obesity after short-term moderate exercise. Methodology: All procedures were approved by the animal use and care committee (Protocol #2019-007). 12 weeks old male Zucker obese rats were randomly divided into a sedentary obese group and an exercise obese group (n=4/group). The exercise consisted of 4 weeks of swimming training for 60min/5days/week. After 48 hours of the last exercise bout, animals were euthanized and both gastrocnemius muscles were isolated. Data are expressed as mean ± standard error of the mean; unpaired t-test was performed for comparison between groups. Results: After four weeks of swimming training, a significant increase in fission was evidenced by changes in phosphorylation of Drp1 and AMPK (1.4 and 1.3, $p<0.01$). Subsarcolemmal mitochondria showed a more organized network in comparison with the sedentary group ($p=0.04$) and a significant increase in gastrocnemius myofiber area ($p=0.03$) was observed. Conclusion: An increase in fission regulated by AMPK might be segregating damaged mitochondria and enhancing its removal while activating mitochondrial biogenesis to ensure restoration of mitochondrial mass by generating a healthier population in the subsarcolemmal region.

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NQ645QK

VCAM-1 expression mediates the protective effect of TNF-α preconditioning against ischemia/reperfusion injury in cultured cardiomyocytes

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Introduction: Tumor necrosis factor-α (TNF-α) has been associated with the development of cardiovascular diseases, including cardiac ischemia/reperfusion (I/R) injury. Low concentrations of TNF-α exert cardioprotective effects, whereas at high concentrations induce cardiomyocyte death by apoptosis. Moreover, TNF-α induces the expression of vascular cell adhesion protein-1 (VCAM-1) in several cell types. Our previous results showed that VCAM-1 is associated with cardiomyocyte survival in a simulated ischemia model. Objective: This work aims to evaluate whether TNF-α preconditioning stimulates cardiomyocyte VCAM-1 expression to protect them against simulated I/R injury. Methodology: Cultured neonatal rat ventricular cardiomyocytes were treated with TNF-α (0 to 500 ng/mL), and cell viability was assessed by MTT assay and trypan blue exclusion. Protein and mRNA levels of VCAM-1 were measured by Western blot and RT-qPCR, respectively. Then, VCAM-1 knockdown cardiomyocytes were pre-treated with 10 ng/mL TNF-α for 24 h and incubated under ischemic conditions for 6 h. Later, the ischemic medium was replaced by DMEM/M199 containing 10% FBS and cardiomyocytes were exposed to normoxia conditions for 16 h. Cell viability was assessed at the end of reperfusion. Results are shown as mean ± SD (n≥3). ANOVA with post hoc test was performed as appropriate, p-value < 0.05 was considered statistically significant. Experimental protocols were approved by the Institutional Committee for Care and Use of Animals from Universidad de Chile. Results: TNF-α treatment (concentrations < 100 ng/mL for 24 h) did not stimulate cardiomyocyte death. Also, TNF-α induced VCAM-1 expression (protein and mRNA) through a transcriptional mechanism in cardiomyocytes. TNF-α preconditioning for 24 h protects cardiomyocytes from simulated I/R injury, and this effect seems to be mediated by VCAM-1. Conclusion: TNF-α preconditioning protects cardiomyocytes against simulated I/R injury, and this effect could be mediated by VCAM-1.
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QN144RQ

Angiotensin II and O2 regulate RECK expression mediating human trophoblast endothelial-like differentiation: potential role in preeclampsia

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Introduction: The placenta is a transient organ that forms during pregnancy to support fetal growth. During human placental development fetal trophoblast cells invade the maternal decidua and remodel the uterine spiral arteries acquiring an endothelial-like phenotype. Appropriate trophoblast behavior is critical to ensure a good pregnancy outcome as inefficient invasion and remodeling of spiral arteries underlie the development of preeclampsia (PE). Reversion-inducing cysteine-rich protein with Kazal motifs (RECK) is conceived as a critical regulator of extracellular matrix remodeling and angiogenesis. Our group has previously shown that RECK regulates trophoblast invasion capacity and that its protein abundance is higher in placentas with the severe form of PE. Angiotensin II (Ang II) and Oxygen (O2) tension are two major factors involved in the regulation of trophoblast invasion. Increased placental Ang II and low O2 levels have been reported in PE, however, whether these factors interact to regulate RECK expression and drive trophoblast endothelial-like differentiation remains unknown. Aim: Determine the effect of Ang II and O2 tension in RECK expression and their role in mediating trophoblast endothelial-like differentiation. Methods: Human trophoblast HTR8/SVneo were treated with Ang II under different O2 tensions. RECK expression was evaluated by Western Blot and qPCR. Transwell invasion assays were performed. Endothelial-like transition was assessed by Endothelial tube formation assay and expression of differentiation markers and evidenced by ImageJ software analysis. This project has the approval of the ethics committee, Universidad San Sebastian, period 2018-2022. Results: Ang II increased RECK expression while low O2 tension reduced RECK expression. Ang II and high O2 tension decreased trophoblast invasion, stimulating endothelial-like differentiation. Statistical comparisons were analyzed using ANOVA and Bonferroni's multiple comparisons test. Data is expressed as mean SD, n=3 for each experiment, P<0.05. Conclusion: Ang II and O2 tension regulate RECK expression and mediate trophoblast endothelial-like differentiation.
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GG651NL

Negative charges at the ion binding site of the AE4 Cl-/cation-HCO3- exchanger affect the HCO3- transport

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Introduction: The Cl-/cation-HCO3- exchanger AE4 (SLC4A9) plays a role in renal NaCl reabsorption, fluid secretion in salivary glands, and pH regulation in tumor cells. Structural data in SLC4 proteins suggest that the ion binding site is in a cavity where half-

helices TM3 and TM10 meet. It has been proposed that the anion binding depends on the positive helical dipoles provided by those helices, as well as the side chains from several residues in this region. Our sequence alignments showed that the proposed ion binding site is conserved in AE4, suggesting that its transport mechanism might depend on the same electrostatic interactions. Objective: To determine the functional consequences of modifying electric charges at the TM3-TM10 region of AE4. Methods: Molecular modeling, site-directed mutagenesis, and HCO3- transport assays using imaging techniques were performed. Results are shown as means ± SEM of 5 independent experiments from at least 3 different transfections. One-way ANOVA followed by Bonferroni´s post hoc test was performed. A significant difference was considered with $p < 0.05$. Results: Our molecular simulations showed that the ion binding site in AE4 is in the TM3-TM10 interphase. Mutation of T448 (TM3) and T756 (TM10) decreased HCO3- transport by ~50 and ~30% respectively, suggesting the role of the hydroxyl groups in the transport cycle. To probe that negative charges in this region might affect the anion transport, we mutated a set of residues by aspartate. Mutants T448D and G449D, decreased HCO3- transport by ~40%, while A755D, T756D, and V757D, decreased transport by ~60, 70, and 40% respectively. Our molecular simulations showed that negative charges at TM3-TM10 interphase modify electrostatic potential and promote structural rearrangements. Conclusions: Our results suggest that introducing negative charges at the TM3-TM10 interphase might modify the local electrostatic potential affecting the anion binding and transport.
Núcleo Milenio de Enfermedades Asociadas a Canales Iónicos (MiNICAD), Ministerio de Economía, Fomento y Turismo, Gobierno de Chile; FONDECYT # 1191868 (SB)

DL516GT

The effect of urea on the Ca2+ signaling in mouse submandibular gland acinar cells

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Introduction: Decreased salivary flow is frequent in chronic kidney disease. Increased levels of plasmatic and salivary urea are common, however, the effect of this metabolite on the molecular mechanisms of salivation is unknown. The main molecular event stimulating saliva secretion is an increase in the intracellular Ca2+ concentration in acinar cells of salivary glands. Previous studies reported an effect of urea in Ca2+ signaling in muscle cells, and decreased insulin secretion in pancreatic β cells. Here, we speculate that urea might affect the Ca2+ signaling in salivary glands. Objective: To determine the effect of urea on the intracellular Ca2+ signaling in mouse submandibular gland acinar cells. Methods: The Ca2+ indicator Fura-2 was used to record intracellular Ca2+ dynamics in isolated mouse submandibular acinar cells. Intracellular Ca2+ mobilization was stimulated with 0,3 µM carbachol (CCh). Controls were compared with cells exposed to 25 or 50 mM urea. Results are shown as means ± SEM of at least 10 independent experiments from at least 4 different mice. The P value was obtained by the Student's t test. All animal procedures were approved by the Animal Care and Use Committee of the Universidad Austral de Chile. Results: We found that the CCh-stimulated Ca2+ response decreased by ~60% in cells exposed to 25 or 50 mM urea. The basal intracellular Ca2+ concentration was not different in controls compared to cells exposed to urea. Moreover, in cells exposed to Ca2+-free bath solutions, the CCh-stimulated intracellular Ca2+ mobilization decreased by ~80% in the presence of urea. On the other hand, the extracellular Ca2+ influx was not different in cells exposed to urea when cells were re-exposed to Ca2+-containing solutions. Conclusions: Our results indicate that urea affects the intracellular Ca2+ release in acinar cells, probably by interfering with the mechanisms of muscarinic stimulation.
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LP393HD

Cardiovascular effect of cannabis sativa oil on hypertensive rats

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Introduction: Cannabis sativa oil has been used for different medical purposes like pain relief or refractory epilepsy treatment. Its main pharmacological components are Δ-9-tetrahydrocannabinol (THC) and cannabidiol (CBD). CBD, through the endocannabinoid-system receptors (CB1 and CB2) has cardioprotective effects against inflammatory and oxidative damage. However, the effect of Cannabis sativa oil on hypertensive cardiac hypertrophy (CH) remains unclear. In CH dysfunctional mitochondria may produce deleterious effects on heart function. Objective: The aim of this study was to evaluate the activation

of the endocannabinoid system attenuates CH and improves myocardial mitochondria function in spontaneously hypertensive rats (SHR). Methods: Three month old male SHR were randomized into treated (TR, n=4) and control (CR, n=4) and Cannabis sativa oil was orally administered for 1 month to TR. We measured CH by weighting and by ultrasound and mitochondrial membrane potential by spectrofluorometry. Data are presented as mean±SEM and Welch's t-test was used for statistical differences ($p<0.05$). Protocols were approved by the Care and Use of Laboratory Animals Committee of our institution. Results: CH, determined by left ventricular weight/tibia length ratio, was reduced by treatment (mg/mm, TR: 28.28±0.58; CR: 32.31±1.1, $p<0,05$). Comparison of cardiac ultrasounds at the beginning and end of treatment showed exclusively in TR: 24.7% reduction in LV mass ($p<0,05$) and a significant decrease in LV wall thickness (from 1,85 ± 0,01 to 1,58 ± 0,02) without changes in LV diastolic dimension and arterial pressure. Mitochondrial membrane potential was improved by treatment (in mV, TR: -165.9±3.05; CR: -150.6±4.47, $p<0,05$). Conclusion: Based on these results we propose that a 1-month treatment with Cannabis sativa oil in SHR is effective to reduce CH and improve the mitochondrial membrane potential, possibly traducing into better mitochondrial function.
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DG893QQ

Fused mitochondrial morphology, and a glycolytic and lipogenic metabolism in metastatic triple-negative breast cancer

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Introduction: Mitochondrial function and morphology have a relevant impact in the metabolic program, the metastasis, the proliferation and the survival of cancer cells. Triple-negative breast cancer (TNBC) is an aggressive cancer with poor prognosis and no-specific treatment. Recently, it has been observed heterogenous metastatic and metabolic properties in TNBC which could be related with their aggressiveness. Nevertheless, the role of the mitochondrial function and morphology in the metastasis and metabolism of TNBC have not been widely explored, which is relevant for the research of new therapies. Objective: This research aimed to explore the differences of gene expression of proteins related to oxidative and non-oxidative metabolism in metastatic and non-metastatic TNBC cell lines, as well their mitochondrial function and morphology. Methods: We explored gene expression by qPCR analysis and, mitochondrial potential and morphology by confocal microscopy in the cell lines MDA-MB-231 (metastatic TNBC), HCC-1395 (non-metastatic TNBC) and the control MCF-12A (immortalized breast cell line). The results were assessed by unpaired Student´s T-Test and one way ANOVA. Results: Genes related to glycolytic metabolism and fatty acids biosynthesis were overexpressed in MDA-MB-231, accompanied by fused mitochondrial morphology and lower activity. In contrast, HCC-1395 presented upregulated expression of mitochondrial biogenesis-related genes and hyperfragmented mitochondria accompanied by higher ROS production. Conclusion: The differences in the mitochondrial activity, the mitochondrial morphology and the metabolism related gene expression found between HCC-1395 and MDA-MB-231, will provide a better understanding of the metastasis process in TNBC and the improvement of the development of specific therapies.
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BD619MT

Cellular mechanisms underlying the low cardiotoxicity of istaroxime

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Introduction: Na+/K+-ATPase (NKA) inhibition induces Ca2+calmodulin kinase II (CaMKII)-dependent cardiomyocyte death and arrhythmias. Novel NKA inhibitor istaroxime presents lower risk of Ca2+triggered arrhythmias by accelerating Ca2+uptake via sarcoplasmic reticulum Ca2+ATPase (SERCA).Objective: to test if istaroxime at therapeutic concentrations impact cardiomyocyte viability and gain insight into its mechanisms. Methods: Experiments were performed in vitro and approved by the Institutional Animal Care and Use Committee of La Plata University. Rat cardiomyocytes were paced at 1 Hz and superfused with ouabain 2 µM or istaroxime 10 µM. Cell shortening was registered by video edge-detection. Cells were cultured at 37° C with and without the drugs, and after 24 hs evaluated morphologically to assess viability. Ca2+spark and wave frequency were measured by confocal microscopy in Fluo-4 loaded myocytes after 1 hour-drug incubation. CAMKII activity (p-CAMKII), phosphorylation of threonine-17 (Thr17) of phospholamban (PLN) and apoptotic index BAX/BCL-2 were quantified by western blot in cell homogenates after 1 hour-drug incubation. Unpaired Student t test and One-way ANOVA followed by Tukey's post-test were used for statistical comparisons of quantitative data and Fisher's exact test to compare incidence of qualitative data between groups. Results: 2 µM ouabain and 10 µM istaroxime showed equivalent inotropic effect (n= 15 and 23). Percentages of cell viability were obtained: Control 52 ± 2.5%; Ouabain 2 µM 33 ± 3%; Istaroxime 10 µM 46 ± 3% (n=7 per group). In contrast to ouabain, istaroxime did not promote significant CaMKII activation or apoptosis (N=4 per group) or increase Ca2+spark and wave frequency, but increased the

proportion of aborted Ca2+waves. This lower wave incidence persists in cells from PLB-KO mice, suggesting that relief on PLB-dependent SERCA inhibition is not the only mechanism underlying lower arrhythmogenesis. Conclusion: istaroxime produces significant inotropic effect without inducing CaMKII-dependent cardiomyocyte death. New insights are provided to explain low arrythmogenesis.

HC584TB

H+ transporters that participate in cell pH regulation in the HT-29 cell line.

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Introduction: The regulation of intracellular pH is important for all cells, since all biological processes are sensitive to intracellular pH. Cell pH regulation was investigated in the HT-29 cell line derived from epithelial colon cancer. Objective: The aim of this work was to functionally determine H+ transporters that participate in cell pH regulation in these cells. Methods: Cell pH was measured by ratiometric fluorescence microscopy using the fluorescent probe 2,7-bicarboxyethyl-5,6-carboxyfluorescein acetoxymethyl ester (BCECF-AM), 12 uM. All experiments were realized in the absence of NaHCO3. Results: Basal pH was 7.17 ± 0.026 (n= 8). After acidification by an ammonium pulse (20 mM, pH 7.4), cell pH recovered toward normal at a rate of 0.2 ± 0.026 pH units/min (dpH/dt). In the presence of HMA (0.1 mM) or HOE-694 (25 uM), NHEs inhibitors, the pH recovery rate was 0.03 ± 0.006 (n=5). When was added Schering (10 uM, H+/K+-ATPase inhibitor) or concanamycin A (0,1uM, V-ATPase inhibitor) or both in presence of HOE-694, the pH recovery rate was 0.02 ± 0.009 (n=5) and 0.008 ± 0.005 (n=5), respectively. Conclusion: these experiments show that Na+/H+, H+/K+-ATPase and V-ATPase transporters participate in cell pH regulation, NHE being the most important.

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HN873TD

Cross-Linking between oxidative stress and circulating extracellular vesicles after tyrosine kinase inhibitor treatment.

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Introduction: Tyrosine kinase inhibitors (TKI) such as Sunitinib (Su), are used as antiangiogenic drugs for cancer treatment and are associated with cardiovascular toxicities (CT) through unknown molecular mechanisms. The circulating extracellular vesicles (VEs) might play a crucial role in the development of these CT produced. Moreover, oxidative stress together with endothelial damage has been purposed as a major contributor to the secondary effects of those drugs. Objectives: The aim of this work was to deepen our understanding of the underlying oxidative stress mechanism involved in the development of arterial hypertension and elucidate the VEs role in this pathophysiology. Methods: All procedures complied with the EU Directive 2010/63/EU and the National (RD 53/2013) guidelines and were approved by the competent Institutional Animal Care and Use Committee (approval reference #08/03/2017/034). Endothelial function in non-treated animal aortas preincubated with VEs from Su-treated animals was performed. Oxidative stress and nitric oxide metabolism, and inflammation markers detection, were carried out in thoracic aortas from male Wistar rats treated with Su for 3 weeks. Results were expressed as the mean ± SEM, n=4-8. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test and considered statistically different at $p<0.05$.Results. Reduced endothelium-dependent vasodilation was observed in normotensive rat aortic rings preincubated with VEs from Su-treated rats, the latter being reverted by an antioxidant substance. Elevation in superoxide anion production and the activity/protein/gene expression of NADPH oxidase isoforms, which was also prevented by isoforms inhibition. Furthermore, Su-treated animals shown a decrease in NO levels, which was reverted before use NOX inhibitors. Conclusions: Our findings in this pilot study open the way to study the molecular mechanism involving the interplay between circulating extracellular vesicles and oxidative stress. Acknowledgments: Ayudas para la Promoción de Empleo Joven e Implantación de la Garantía Juvenil en I+D+i 2017-2020 (PEJ2018-004474-A).

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DJ767LQ

Sex dimorphism in placenta expression of Fatty Acid Transporters in women with pregestational obesity and in response to maternal DHA supplementation

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Introduction: Pregestational obesity (PO) has detrimental consequences for maternal, and fetal development. Changes in the expression of genes involved in fatty acids (FA) transport have been described in placenta from women with PO, including Docosahexaenoic acid (DHA), an essential FA key for fetal development. However, the role of neonatal sex in these changes in placental gene expression in women with PO and in those supplemented with DHA is unknown. Objectives: We aimed to determine sex-difference in the mRNA expression of the principal FA transporters in PO placenta and the effect of maternal DHA supplementation. Methods: Pregnant women with PO were recruited (<15 weeks) and randomized to receive 200 (PO-200) or 800 (PO-800) mg/day of DHA, until delivery. The protocol was approved by the institutional ethics committee and all patients signed an informed consent. A group of normal-weight women were recruited as a reference group (NW). At birth, the placentas were dissected, and trophoblast fragments were stored (RNAlater/-80°C) for qPCR analysis for FAT/CD36, FABP3 and FABP4. Statistical analyses were performed by Student's unpaired t-test and Kruskal-Wallis. Results: When separated by sex, female placentas show an increased expression of FAT/CD36 (1.5-fold) and FABP3 (2-fold) in PO-200, which was reverted in PO-800. Male placentas show an increased expression of FABP4 (2.5-fold) in PO-200 without changes in PO-800. In female placentas FABP3 and FABP4 expression correlated to maternal and cord cholesterol, respectively. In male samples FABP3 expression correlated to maternal and cord cholesterol and adiponectin, whereas FABP4 correlated with maternal BMI and placental monounsaturated, saturated and total placental FA levels. Conclusion: These results suggest a sexual dimorphism in the FA transporters gene expression in placenta. Maternal PGO affects the expression of FAT/CD36 and FABP3 in girls and FABP4 in boys. Only the female-dependent changes in gene expression were modulated by maternal DHA supplementation.
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JN121BF

Effect of Oral Magnesium Gluconate supplementation in Salt-loaded Pregnant Rats on the levels of Lipid Peroxidation and Ca-ATPase Activity in Placental Homogenates

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Introduction: Preeclampsia is associated with an increase of the levels of lipid peroxidation in the placenta and diminution of Ca-ATPase activity in this tissue. The standard treatment for preeclampsia has been intravenous magnesium sulfate (MgSO4). Currently, other oral magnesium salts are being studied, e.g. magnesium gluconate (MgC12H22O14, in this abstract asMgGl2), which has greater antioxidant capacity than MgSO4. Placental homogenates from an animal model of preeclampsia (salt-loaded pregnant rats during the last week of pregnancy) show an increase in lipid peroxidation, which is associated with a decrease in Ca-ATPase activity. In addition, in vitro incubation of placental homogenates from these rats with MgGl2 is able to reverse the increase of lipid peroxidation. Objective: To evaluate the in vivo effect of MgGl2 on lipid peroxidation and Ca-ATPase activity in placenta homogenates from salt-loaded pregnant rats. Methods: After 14 days pregnancy, healthy rats were given to drink either tap water (control pregnant rats) or 1.8% NaCl solution (salt-loaded pregnant rats) with and without 3g/kg/day MgGl2. At the end of the pregnancy the placentas were removed, homogenized and assayed for lipid peroxidation (TBARS) and Ca-ATPase activity. Statistical analysis was performed by one-way ANOVA with a post-test Student-Newman-Keuls. The results are expressed as means ± standard error with minimum n=3. A statistical significance level of $p \leq 0.05$ is accepted. The protocol of the study was approved by the Bioethics Committee for animal studies at IVIC (COBIANIM) Results: Oral MgGl2 is able to avoid the increase of TBARS and the diminution of Ca-ATPase activity in the placenta of salt-loaded pregnant rats. Conclusion: Oral MgGl2 protects pregnant rats from lipid peroxidation in the placenta, which could be responsible of the diminution of Ca-ATPase activity during salt overload. This magnesium salt could be an oral alternative to replace MgSO4 as a treatment for preeclampsia.
This work was supported by DR's PhD Program in Biochemistry at IVIC.

FL353QD

Effect of Oral Magnesium Gluconate Supplementation in Salt-loaded Pregnant Rats on Osmotic Fragility, Lipid Peroxidation and Ca-ATPase Activity in Red Blood Cells Ghosts

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Introduction: The standard treatment for preeclampsia has been intravenous MgSO4. Other magnesium salts have been used as alternative treatments during preeclampsia. One of these salts is magnesium gluconate (MgC12H22O14, in this abstract MgGl2), which has been shown to possess higher antioxidant capacity than MgSO4. In vitro treatment with MgGl2 was able to reverse the increased lipid peroxidation level and the diminished Ca-ATPase activity in red blood cell ghosts from preeclamptic patients (1). An animal model of preeclampsia (salt-loaded pregnant rats during the last week of pregnancy), shows an increase of osmotic fragility in red blood cells as well as an increase of their lipid peroxidation and a diminution of their Ca-ATPase activity. Objective: To evaluate the in vivo effect of MgGl2 on osmotic fragility, lipid peroxidation and Ca-ATPase activity in red blood cell ghosts from salt-loaded pregnant rats. Methods: After 14 days pregnancy, healthy rats were given to drink tap water (control pregnant rats) or 1.8% NaCl solution (salt-loaded pregnant rats) with and without 3g/kg/day MgGl2. At the end of the pregnancy, blood was drawn from left ventricle and osmotic fragility was assayed. Red blood cell ghosts were prepared and assayed for TBARS and Ca-ATPase activity. Statistical analysis was performed by one-way ANOVA with a post-test Student-Newman-Keuls. The results are expressed as means ± standard error with minimum n=3. The study was approved by the Bioethics Committee at IVIC (COBIANIM). Results: Oral MgGl2 produces a statistically significant diminution of the osmotic fragility of red blood cells, as well as a diminution of the TBARS and a concomitant increase of Ca-ATPase activity in these membranes in salt-loaded pregnant rats. Conclusion: Oral MgGl2 stabilizes the red cell membrane of salt-loaded rats, diminishes their level of lipid peroxidation and increases their Ca-ATPase activity. Oral MgGl2 is a promising treatment for preeclampsia.
This work was supported by DR's PhD Program in Biochemistry at IVIC.

MD798JN

Immune Check Point Inhibitors (ICIs) induce myocarditis in a heart failure model

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Introduction: Several immune-related adverse events (irAEs) have been described for combined ICIs therapy (anti-CTLA-4 + anti-PD-1). Among ICIs-related irAEs, Myocarditis can become lethal. Here, we showed that ICIs induce Myocarditis in an Angiotensin II-derived Heart failure model. Objective: The purpose of this study was to determine the myocarditis development. In a Heart failure model after ICIs therapy. Methods: Angiotensin II was used to generate the chronic injury (Chr. Inj.). Then, combined ICIs therapy was administered weekly. Four weeks after the treatments, we analyzed: i) the survival time of mice, ii) the recruitment and phenotype of infiltrating immune cells to the heart iii) fibrotic changes, iv) expression of remodeling tissue markers and local or systemic inflammatory cytokines. ANOVA was used for the statistical analysis (mean with SEM); all procedures were approved by the Institutional Animal Use and Care Committee (2019-016).Results: After weekly doses of ICIs in a Chr. Inj. mice, the ICIs reduced the survival and induced myocarditis. The infiltrated cells in heart were characterized as mainly T CD3+ CD4+ cells. Conclusion: We found that preexisting damage is necessary in order to promote ICIs-induced myocarditis and the increase of the mortality. Furthermore, the infiltrate is mainly due to the CD3+ CD4+ cell recruitment.
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CB661BT

Acute effect of FGF21 on GLUT4 translocation, glucose uptake and Ak phosphorylation in L6 muscle cell line

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Introduction: Glucose homeostasis is regulated by the interaction of several organs, including skeletal muscle. Glucose uptake in skeletal muscle is a diffusion process facilitated by the action of the glucose transporter type 4 (GLUT4) in response to stimuli such as insulin or muscle contraction. L6-GLUT4myc is a cell line used in research to study glucose uptake and translocation mechanisms of GLUT4 in skeletal muscle. Recently, it has been described that fibroblast growth factor 21 (FGF21) induces GLUT4-dependent

glucose uptake in adult skeletal muscle fibers. However, the acute effect of FGF21 on L6-GLUT4myc cell line has not been described. PURPOSE: "To assess the acute effects of FGF21 on glucose uptake, GLUT4-translocation and phosphorylation of Akt at serine 473 in the skeletal muscle cell line L6-GLUT4myc". Methods: L6-GLUT4myc myoblasts were cultured and differentiated into myotubes before to be stimulated with 100 ng/mL of FGF21 for 20 minutes. Insulin was used as a positive control. GLUT4myc exposure in surface on plasma membrane were estimated by an optical detection assay, employing the reagent O-phenylenediamine (OPD). To assess glucose uptake, the fluorescent deoxyglucose analogue 2-NBDG was used. Western blot assays were performed to determine Akt-serine 473 phosphorylation and protein content of FGFR1 and β-Klotho (KLB). One-way ANOVA for nonparametric Kruskal-Wallis data with post hoc multiple Dunn comparisons were performed. Experiments included a sample size of 4-5. Results were considered as mean and ± SD. Results: FGF21 did not induce changes in GLUT4 surface exposure, cortical actin remodeling nor Akt serine 473 phosphorylation in L6-GLUT4myc myotubes. Additionally, a low relative expression of the FGFR1 receptor was observed in L6-GLUT4myc myotubes. Conclusion: L6-GLUT4myc myotube cell line does not respond to an acute FGF21 stimuli, suggesting that it is not a suitable experimental model for FGF21-induced glucose uptake in muscle cells.
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GK828MC

Changes in placental mitochondrial function are associated with fetal weight variation within the litter of mice and the nature of these changes depend on fetal sex

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Introduction: The placenta is essential for fetal growth. In turn, placental growth and function depend on energy that is primarily generated by mitochondria. Placental mitochondria have been shown to alter their function both during normal gestation and in response to adverse conditions. However, we lack knowledge on the relationship between placental mitochondria function and fetal weight in late gestation. Objective: To investigate whether changes in mitochondrial function in the transport zone of the mouse placenta (labyrinth zone; Lz) relate to fetal weight variations for each sex within the litter. Methodology: Pregnant females (n=7) were killed on gestational day 18. Fetuses and placentas were dissected and weighed. Lz was microdissected and half was cryo-preserved for assessment of mitochondrial function using high-resolution-respirometry (HRR) or snap-frozen for gene expression analysis using qPCR. HRR and qPCR analysis of the Lz was performed on the heaviest and the lightest fetuses per sex in each litter. Data were analyzed by paired t-test within each sex. Experiments were performed in accordance with UK Animals Scientific Procedures Act. Results: Lz oxygen consumption at mitochondrial complexes I and II was greater for the placentas supporting the lightest-female fetuses, compared to those supporting the heaviest. However, no differences in Lz mitochondrial respiratory capacity were found in placentas supporting the lightest versus heaviest-male fetuses. The expression of genes involved in mitochondrial biogenesis (eg. Nrf1) and fission (eg. Drp1) was lower in the Lz of lightest versus heaviest-female fetuses. In contrast, expression of the mitochondrial-related gene, Pparα was greater in the lightest compared to heaviest-male fetuses. Conclusion: Changes in mitochondrial function and related genes were associated with fetal weight variations within the litter, but the nature of these changes depends on fetal sex. These data may be useful for tailoring the development of treatments to improve fetal outcomes of males and females.
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LK211BB

Adipose tissue–derived exosomal microRNA (exomiR) regulates placental glucose uptake in gestational diabetes mellitus (GDM) pregnancies

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The aim of this study was to profile the adipose tissue exomiRs in patients with normal glucose tolerant (NGT) and gestational diabetes mellitus (GDM). Omentum were obtained from BMI-matched NGT (n=10) and GDM (n=10) women at delivery (>37 weeks gestation). Isolated exosomes were characterised based on size distribution and protein enrichment. Illumina TrueSeq Small RNA kit was used to construct a small RNA library. The resulting sequencing FASTQ files were analysed using miRDeep2 to identify both known and novel miRNAs. A selected exomiR mimic was overexpressed in the human primary trophoblast (PHT) cells and its effect on glucose uptake was evaluated using 2-NBDG. Statistical differences between groups were identified by post hoc analyses Dunnett's tests where the data distribution approximates normality or by Mann-Whitney U-test for distribution independent data analysis. This study was approved by the Human Research Ethics Committees of the University of Queensland (HREC/11/QRBW/342), and the Mercy Hospital for Women (HREC R10/16 and R04/29) Ethics Committees We identified a total of

1209 miRNAs with 18 miRNAs were significantly differentially expressed in GDM exosomes. Comparatively, 12 miRNAs were upregulated while 6 were downregulated in the exosomes from GDM pregnancy compared to normal pregnancy. Consequently, has-miR-515-5p was selected to be investigated in further functional studies, following validation of its expression using real-time PCR. The QRT-PCR analysis of placental tissue showed a significant increase in the expression of GDM compared to NGT placenta. In PHT cells, the overexpression of miR-515-5p significantly stimulated the glucose uptake ($p<0.0001$) compared to the control group. The proteomics analysis of miR-515-5p overexpressed PHT cells showed differential expression of proteins that are associated with glycolysis. These findings suggest adipose tissue exosomal miR515-5p mediated placental glucose uptake could contribute to an excessive placental nutrient transfer GDM, which could instigate consequences, such as fetal overgrowth.
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HN189HS

Maternal-fetal adaptation of placental cells in gestational diabetes results in distinct extracellular vesicle protein and miRNA profiles similar to the cell of origin

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Exosomes are membrane-bound extracellular vesicles secreted from a range of cells including human placental cells and contain specific cargo such as proteins, and miRNAs. The aim of this study was to determine the miRNA and protein profile in primary human trophoblast (PTH) and their secreted exosomes obtained from placentae from women with normal glucose tolerant (NGT) and gestational diabetes mellitus (GDM). Exosomes were isolated from PTH by differential centrifugation and size exclusion chromatography and characterised by size distribution, abundance of exosomal proteins, and morphology. We used quantitative proteomic analysis and miRNA sequencing to determine the protein and miRNA profile in exosomes and their originated PTH. Statistical differences between groups were identified by post hoc analyses Dunnett's tests where the data distribution approximates normality or by Mann-Whitney U-test for distribution independent data analysis. This study was approved by the Human Research Ethics Committees of the Royal Brisbane and Women's Hospital and the University of Queensland (HREC/11/QRBW/342), and the University of Concepcion (002373) Ethics Committees. A total of 34 proteins were significantly different in PTH from GDM compared with NGT. Similarly, a specific set of proteins were significantly different in PTH exosomes from GDM compared with NGT. We identified a set of miRNAs (miR-181, miR-151, miR-10a, miR-744, miR-1468 and miR-4507) which their expression varied in a consistent pattern in PTH and their secreted exosomes in GDM compared with NGT. Interestingly, a specific set of proteins and miRNAs were selectively enriched with exosomes and compared with their PTH of origin in NGT and GDM indicating a specific packaging of proteins and miRNAs into exosomes. Bioinformatics analysis showed that the top canonical pathway associated with these miRNAs were PI3/AKT signalling and glucose metabolism/insulin resistance, respectively. This data supports the hypothesis that exosomes are “fingerprints” of the releasing cells and their metabolic status.
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KR274TF

Combining discovery and targeted proteomics reveals specific extracellular vesicles proteins from placental cells under different physiological conditions

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Introduction: Hyperglycaemia and inflammation are risk factors for metabolic complications during pregnancy. Objective: The aim of this study was to quantify a set of proteins involved in the biogenesis, trafficking, and uptake of extracellular vesicles (EVs) in placental cells in response to changes in the microenvironment milieu. Methods: Placental cells were incubated in the presence of forskolin with D-glucose (5 mM or 25 mM), insulin (1nM), LPS (0-10 mg/ml) and TNF-a (0-20 ng/ml) for 48 hours. Cells were used to develop peptide-based assays for protein quantification, which are incorporated into a method based on a liquid chromatography-multiple reaction monitoring mass spectrometry (LC-MRM/MS) to quantify 111 proteotypic peptides from 37 proteins that have been associated in the biogenesis, trafficking, and uptake of EVs. Statistical differences between groups were identified by Mann-Whitney U-test for distribution independent data analysis. This study was approved by the Human Research Ethics Committee of the University of Queensland. Results: Differential changes in the release of different populations of EVs in response to glucose, insulin, LPS, and TNF-a were observed. High glucose induces the release of EVs <50nm, and >200nm, and the effect abolishes in the presence of insulin. Interestingly, high glucose and insulin decrease the release of EVs 150-200 nm, and EVs 50-150 nm, respectively. The effect of LPS on the release of EVs from placental cells was a size-depend manner with a maximum effect in EVs of >200nm. TNF-a increases the release of EVs in size and concentration-depended manner with a maximum effect on EVs >200nm and 2 ng/ml. Changes in the release of exosomes were associated with a differential abundance of proteins associated with ESCRT machinery. Conclusion: The effects of extracellular milieu on placental-derived EVs release may be

recapitulated and is of clinical relevance in vivo in association with hyperglycemia (glucose and insulin), infection (LPS), and inflammatory (TNF-a) conditions.
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LT187MP

Effect of alliin in adipocyte gene expression from two obesity models: in vitro and in vivo

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Introduction: The metabolic stress in adipocytes and adipose tissue itself is one of the main causes of the chronic low-grade inflammation observed in obesity. The research of molecules with beneficial effects aims to offer an alternative adjuvant treatment for this world health problem. Alliin, an organosulfurized compound from garlic, has been attributed with different beneficial properties, however, its effects on obesity have not been broad studied. As it is well known, obesity is a condition that involves complex metabolic pathway interactions, therefore it is important to carry out wide approaches for the analysis of the phenomenon, preferably of the "omic" type. Objective: To identify possible therapeutic targets and signaling pathways involved in metabolically stressed adipocytes by analyzing gene expression profiles by microarrays in 2 obesity models: in vitro and in vivo. Methods: Two different models were compared: alliin pre-incubated 3T3-L1 adipocytes stimulated with LPS, and diet-induced obesity C57BL/6 mice with 3.5 weeks alliin treatment. Each experiment had its control group (Ethics Committee CI-012108). Microarrays test results, a Z score ≥1.6 was considered significant for the cluster analysis on the DAVID platform. Results. The main functional categories of genes affected in common for both models were cytoskeleton and microtubules, protein transport, transcription regulation, zinc fingers and metal-binding proteins. Conclusions: Alliin has a consistent effect at the cellular (in vitro) and tissue (in vivo) level, acting mainly on the regulation of the transcription of cytoskeletal proteins, cellular transport, and protein secretion. The biological implications of this genes indicate possible modifications in the structure and cellular profile of adipose tissue. These results allowed us to identify clusters of genes for future translational studies to define the possible therapeutic effect of alliin against metabolic alterations in obesity.
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TK756JF

A retinoprotective tool against ocular oxidative stress induced by arterial hypertension: Wild olive (Acebuche) oil-enriched diet.

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Introduction: Oxidative stress plays an important role in the pathogenesis of ocular diseases, including hypertensive eye diseases. The beneficial effects of olive oil on cardiovascular diseases might rely on minor constituents. Currently, very little is known about the chemical composition and/or therapeutic effects of the cultivated olive tree's counterpart, wild olive (also known in Spain as acebuche—ACE). Objectives: Here, we aimed to analyze the antioxidant and retinoprotective effects of ACE oil on the eye of hypertensive mice made hypertensive via administration of NG-nitro-L-arginine-methyl-ester (L-NAME), which were subjected to a dietary supplementation with either ACE oil or extra virgin olive oil (EVOO) for comparison purposes. Methods: Experiments were conducted in accordance with the EU Directive 2010/63/EU and the national (RD 53/2013) guidelines for the care and use of laboratory animals, and was approved by the competent Institutional Animal Care and Use Committee (approval reference #13/03/2019/031). Deep analyses of major and minor compounds present in both oils was accompanied by blood pressure monitoring, morphometric analyses, as well as different determinations of oxidative stress-related parameters in retinal layers, focusing in NADPH system, ROS levels, antioxidant enzyme profile and NO metabolism. All results are presented as means ± SEM. One-way ANOVA followed by a post-hoc Tukey's multiple comparison test were performed with GraphPad InStat Software, and differences were considered statistically different at $p < 0.05$. Results: Aside from its antihypertensive effect, an ACE oil-enriched

diet reduced NADPH oxidase activity/gene/protein expression (with a major implication of NADPH oxidase (NOX) 2 isoform) in the retinas of hypertensive mice. Supplementation with ACE oil in hypertensive animals also improved alterations in nitric oxide bioavailability and in antioxidant enzyme profile. Conclusion: Interestingly, our findings show that the use of ACE oil resulted in better outcomes, compared with reference EVOO, against hypertension-related oxidative retinal damage
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TK139PF

Changes in DNA Methylation Landscape in Monocytes of the Offspring of Women with Pregestational Obesity supplemented with DHA during pregnancy.

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Background: Pregestational obesity (PGO) has been associated with an impaired offspring's postnatal innate immune system among other diseases. Supplementation with Omega-3 long-chain fatty acids (e.g. Docosahexaenoic acid, DHA) during pregnancy has been shown to have maternal anti-inflammatory properties. How this intervention affects the methylation patterns in monocytes of the offspring of women with PGO is still unknown. Aim: This study aimed to describe the effect of DHA supplementation during pregnancy on the methylome of monocytes of the offspring of women with PGO. Methods: In a randomized-controlled study (NTC02574767), which was approved by the Institutional Ethics Committee of INTA, Universidad de Chile, 1000 women with PGO (BMI ≥30 kg/m2 in the 1st trimester) were randomized to supplementation with DHA during pregnancy: with a normative dose (200 mg/day, PGO-200) or with 800 mg/day (PGO-800). Normal weight women (NW) participated as a reference group. Cord blood monocytes (CBM) were isolated by adhesion, genomic DNA isolated and bisulfate treated. Epigenome Wide Association Study (EWAS) was performed by EPIC 850K Array, Illumina® for PGO-200 (n=18), PGO-800 (n=20), and NW (n=20). The data was analyzed using the ChAMP Pipeline. Results: Preliminary results from the CBM methylome showed no changes between NW compared to PGO-200. However significant differentially methylated sites (DMS) were found in 14390 genes in the PGO-800 compared to NW. These DMS were found mainly in the gene body and intergenic regions of non-CpG island sites. We identified DMS located on genes associated with immune response (e.g. MCP1 and PPARγ) and metabolism regulation (e.g. INS and LEP), which were characterized by higher levels of methylation in monocytes from PGO-800 compared to those from NW. Conclusions: DHA supplementation during pregnancy modulates changes in the DNA methylation landscape of monocytes of the offspring of women with PGO, which could regulate the immune response in these neonates.
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LH279FR

Endoplasmic Reticulum Stress through ATF6a pathway is associated with an inflammatory response in endometrial stromal cells: potential regulation by miRNAs

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Introduction: During decidualization, endometrial stromal cells undergo endoplasmic reticulum stress (ERS) and unfolded protein response (UPR), which will allow them to expand their endoplasmic reticulum with the corresponding machinery for protein folding. These processes are directed by miRNAs that regulate the expression or stability of their transcription factors. Objectives: we focus on the role of ERS/UPR during decidualization to induce a physiological sterile inflammatory response and whether it might be regulated by miRNAs. Methods: We used an in vitro model of decidualization represented by human telomerase-immortalized endometrial stromal cell line St-T1b ; and endometrial biopsies from patients with recurrent spontaneous abortions (RSA) and recurrent in vitro fertilization failures (RIF). Statistical analysis: Student's t test. Mean±S.E.M. of at least 4 independent experiments. The study was approved by the respective local ethic committee (Friederich Schiller Universität). Results: We evaluated the expression of the ERS-sensor ATF6 and the UPR marker, CHOP. Both markers increased in decidualized cells, and Tg (ERS inducer) induced even higher levels in comparison with non-decidualized cells. Then, we evaluated the modulation of TXNIP, a link between the ERS-pathway and inflammation. TXNIP increased in decidualized cells, and also the inflammasome NLRP3 and IL-1b expression. Then, using an in silico analysis using miRTarBase v8.0 we selected two miRNAs able to regulate the ERS and UPR pathways: miR-193b-3p and miR-21-5p. Both miRNAs significantly decreased in non-decidualized cells in the presence of Tg. Finally, we studied the expression and localization of miRNAs through an In Situ Hybridization (ISH) technique in endometrial

samples. Both miRNAs were expressed in stromal cells and endometrial glands in samples from RSA and RIF patients at similar levels. Conclusion: The present results suggest that decidualization in St-T1b cells is accompanied by an ERS and UPR associated with a sterile inflammatory response potentially regulated by miR-193b-3p and miR-21-5p.
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SS341DD

Insulin reverses the increase in L-arginine/nitric oxide signalling via p44/42mapk activation in human umbilical vein endothelium from women with gestational diabetes mellitus under insulin therapy

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Gestational diabetes mellitus (GDM) associates with foetoplacental endothelial dysfunction. Women with GDM treated with diet (GDMd) that show hyperglycaemia are passed to insulin therapy (GDMi). HUVECs from GDMi show increased L-arginine transport and nitric oxide synthesis via the endothelial nitric oxide synthase. GDMi-associated alterations in HUVECs are reversed by exogenous insulin. Insulin triggers activation of 44 and 42 kDa mitogen-activated protein kinases or protein kinase B/Akt via activation of insulin receptor A or B, respectively. Objective: To determine whether activation of p44/42mapk and Akt by insulin is required to reverses the GDMi-associated endothelial dysfunction. Methods: The study has the approval of the ethics committee of Pontificia Universidad Católica de Chile. HUVECs were isolated from full-term normal, GDMd, or GDMi pregnancies. Activator phosphorylation of p44/42mapk, Akt, and eNOS was evaluated by Western blot. IR-A and IR-B mRNA expression was measured by RT-qPCR. L-Arginine transport kinetics (0-1000 µmol/L, 1 min, 37ºC) was determined in Krebs solution and NO level was measured in DAF-FM (5 µL, 45 min). Experiments were performed in the absence or presence (8 h) of 1 nmol/L insulin, 1 nmol/L Akt inhibitor IV, and 1 nmol/L PD-98059. Results: HUVECs from GDMi showed higher ($P<0.04$, unpaired one-way ANOVA) IR-A mRNA expression (2.6 ± 0.48 fold) compared with normal pregnancies. Phosphorylation of p44/42mapk was higher in GDMi (1.7 ± 0.04 fold) and GDMd (2.4 ± 0.06 fold) compared with normal pregnancies. Without insulin, PD-98059 but not Inh IV reversed the GDMi-increased maximal L-arginine transport capacity. GDMi and GDMd-increased NO level, eNOS protein abundance and activator phosphorylation, and hCAT-1 mRNA expression were unaltered by these inhibitors. Insulin blocked the GDMi and GDMd-increased NO level, eNOS protein abundance and activator phosphorylation, and hCAT-1 mRNA expression, an effect blocked by PD-98059. Conclusion: Insulin beneficial effect on HUVECs from GDMi requires p44/42mapk activity.
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TT665QH

Role of cardiac VCAM-1 in experimental diabetic cardiomyopathy

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Introduction: The prevalence of obesity, insulin resistance, and diabetes mellitus has increased during the last years. Diabetic cardiomyopathy (DCM) is a clinical condition that includes all the diseases named and has detrimental effects on cardiac function. However, the molecular mechanisms associated with its development remain still poorly understood. VCAM-1 is an endothelial transmembrane sialoglycoprotein involved in the transmigration of inflammatory cells from the blood to the tissues. However, the role of VCAM-1 in diabetic cardiomyopathy induced by a high-fat diet (HFD) is not fully understood. Objective: To investigate the changes in the expression of cardiac VCAM-1 in a DCM experimental model. Methods. We used male C57BL/6 mice feed with HFD or control diet for 25 weeks. We measured morphometric and myocardial functional parameters, including body and heart weight, fractional shortening (FS%), E/A, cross-sectional area of cardiomyocyte, glucose tolerance test, and cardiac protein levels of VCAM-1 by Western blot. Additionally, we started to use a cardiomyocyte-specific knockout VCAM-1 mice. Their gene-phenotypes were assessed by PCR and glucose tolerance test was also measured. Differences between groups were tested by t-test or 2 way-ANOVA, $p< 0.05$ was considered statistically significant. The experiments was approved by 20387-CYQ-UCH bioethical protocol. Results: The results showed that animals feed with HFD for 25 weeks increased body and heart weight, E/A parameters in heart, cardiomyocyte cross-sectional area, the area under the curve in glucose tolerance test and a decrease in FS%. All these data agree with DCM phenotype. Also, we found that VCAM-1 protein levels were also increased. Interestingly, in mice

feed with HFD for 5 weeks, cardiomyocyte specific KO-VCAM-1 mice showed an improve of glucose tolerance in comparison with f/f mice. Conclusion: The results suggest that cardiac VCAM-1 expression is increased in DCM and could mediate the impairment in glucose metabolism triggered by the HFD.
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LP419BG

The early development of cardiac hypertrophy, A ROS Consequence?

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Introduction: Obesity-induced oxidative stress has been described as a major player in the progress of cardiac hypertrophy but the redox status of the heart at the early beginning of the pathology has not been fully investigated. We showed previously that NOX4, a ROS generating enzyme, increases in the hearts of mice fed with a high fat diet for 8 weeks. Aim: To investigate the redox status of the heart in obese mice that present early signs of cardiac hypertrophy. Methods: C57BL/6 mice were fed with HFD (60% calories from fat) for 12 weeks. We determined the expression of NOX4, hypertrophic markers and oxidative stress markers such as lipid peroxidation, protein carbonylation and the redox couples NADH/NAD+, NADPH/NADP+ and GSH/GSSG. Finally, we studied the expression of the enzymes: Catalase, Glutathione Peroxidases, Glutathione Reductase and Superoxide dismutase. Results are shown as mean ± SEM (n=6-12) and analyzed by Student's t-test. Ethical approval: CBA 0819 Universidad de Chile. Results: After 12 weeks of HFD, mice were obese, had an increase in heart weight/tibia length ratio (12%, p<0,001) and showed an increase in the expression of ß-MHC (41% p<0.05)), BNP (53% p<0.001) and RCAN1.4 (70% p<0.001) mRNAs. We also found an increase of 75% in NOX4 (p<0.01) but no change in protein carbonylation and a 30% decrease of TBARS (p<0.01). As for the redox couples, there was a 28% decrease in NADH/NAD+ (p<0.01) ratio but a 95% increase in GSH/GSSG (p<0.01) with no changes in NADPH/NADP+ (p=6718). The expression of all antioxidant enzymes increased: Catalase (20%, p<0.01), GPx4 (28%, p<0.05), GR (38%, p<0.01), SOD1(32%, p<0.05) and SOD2(18%, p<0.05). Conclusions: These findings suggest that despite NOX4 increase, there is a reductive stress in cardiac tissue at the early beginning of HFD induced obesity. Acknowledgments: This work was supported by FONDECYT Grant 1160704 to PD and Doctoral fellowship 21160378 to YUM.
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RL735FC

Insulin reverses the gestational diabesity-increased basal pHi via a NHE1 activity-independent mechanism in human umbilical vein endothelial cells

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Introduction: Human umbilical vein endothelial cells (HUVECs) from gestational diabetes mellitus (GDM) show alkaline intracellular pH (pHi) due to increased activity of Na+/H+ exchanger 1 (NHE1). However, insulin treatment effect on NHE1 in HUVECs from GDM is unknown. Objective: To determine whether insulin modulates the pHi in HUVECs from GDM depending on pre-pregnancy maternal weight. Methodology: HUVECs were collected from the Clinical Hospital CHRISTUS-UC (Chile). The study conformed to Declaration of Helsinki. Study groups: Normal pregnancies with normal weight (Nnw, n=9), overweight (Now, n=4), or obese (Nob, n=3); GDM with normal weight (GDMnw, n=4), overweight (GDMow, n=4), or obese (GDMob, 'gestational diabesity', n=5). The pHi was measured in cells loaded with BCECF-AM (12 µmol/L, 10 min) exposed to NH4Cl (20 mmol/L). Basal and pHi recovery rate (dpHi/dt) were in presence of insulin (1 nmol/L, 8 h), 5 µmol/L hexamethylene amiloride (HMA, NHEs inhibitor) and 0.1 µmol/L zoniporide (Zn, NHE1 inhibitor). Results: Basal pHi was higher (P<0.05, unpaired one-way ANOVA) in GDMnw (8.23±0.10), GDMow (7.95±0.31) and GDMob (7.54±0.14) vs Nnw (7.06±0.06) or Now (7.03±0.06) but similar to Nob (8.07, 7.98). Basal pHi was unaltered by insulin in Nnw (7.13±0.05) but reduced in Nob (7.18±0.04). Insulin reduced the pHi only in GDMob (7.21±0.14). Without insulin the dpHi/dt was similar in Nnw (0.0043±0.0012 pHi units/10 s), Now (0.0073±0.0030) and Nob (0.00047, -0.00064). There was not pHi recovery in from GDMnw. The dpHi/dt was absent in GDMnw compared with Nnw (0.0043±0.0012); however, it was higher in GDMow (0.0186±0.0007) vs Now (0.0073±0.0030). Zn blocked the GDMow-increased dpHi/dt to values in Now and Nnw. Insulin unaltered the dpHi/dt without Zn; however, increased the dpHi/dt in

GDMow with Zn. Conclusion: Insulin modulates in a differential manner the pHi in HUVECs from gestational diabesity via a NHE1 activity-independent mechanism compared with GDMnw and GDMow.
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BM723DP

Identify of mitochondrial DNA fragments in plasma of children with obesity and metabolic syndrome and its relationship with pro-inflammatory cytokines increase.

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Introduction: It has been described during obesity and metabolic syndrome, the production of ROS increases due to excess in fatty acids. The high ROS production oxidizes mitochondrial DNA (mtDNA), causing its fragmentation and subsequent release. Previous studies demonstrated when H2O2 was added to isolated rat kidney mitochondria, mtDNA was fragmented, with the MTND3, MTCytB and MTCO1 subunits released through the mitochondrial permeability transition pore to cytosol and plasma. Once in plasma, mtDNA can act as DAMP and bind to Toll-like-9 receptor on circulating leukocytes and activate NF-κβ which promotes the synthesis of cytokines, or bind the NLRP3 receptors and form the inflammasome which activates caspase-1 and, in turn, promotes the activation of cytokines, generating a chronic inflammatory response. Objective: To analyze specific fragments (MTND3, MTCytB and MTCO1) of mtDNA and their relationship with pro-inflammatory cytokines levels in children with obesity and metabolic syndrome. Material and Methods: 40 children with obesity and 40 children with metabolic syndrome were included in the study. Plasma MTND3, MTCO1 and MTCytB fragments levels were evaluated by real time-PCR. Cytokines were measured with flow cytometry. Cytokines were measured with flow cytometry. Spearman correlation was used for the correlation analysis and was performed in Prism. $p<0.05$ was considered statistically significant. Approvals were obtained from the Ethics and Research Committees of the School of Medicine Tecnologico de Monterrey. All legal guardians gave their written informed consent. Results: We found a significant correlation between plasma levels of MTCO1 and IL-1β in obesity($r=0.313$, $p=0.02$), meanwhile in MetS there is a strong significant correlation of MTCO1 levels with IL-18 in those patients with 4 or more factors($r=0.748$, $p=0.003$).Conclusion: MTCO1 could trigger IL-1β expression in early inflammation stages due to ROS production, process which can be regulated by a compensatory mechanism during the inflammation progress, and finally lead to IL-18 synthesis.
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QM435TS

Syncytiotrophoblast-derived extracellular vesicles from normal and preeclamptic pregnancies are internalized and have different impacts on nitrosative stress in human umbilical vein endothelial cells

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Introduction: Preeclampsia (PE) is a pregnancy disorder occurring in ~7% of all pregnancies that is characterized by new-onset hypertension after 20 weeks of gestation. Women with PE present with vascular endothelial dysfunction, contributing to the development of hypertension. It is thought that a dysfunctional placenta could contribute to endothelial dysfunction by releasing higher levels of syncytiotrophoblast-derived extracellular vesicles (STBEVs) in the maternal circulation. It has been shown that STBEVs from normal pregnancies (NP) impair endothelial function. However, although recent literature shows PE-STBEVs differ in composition from NP-STBEVs, the effects of NP- versus PE-STBEVs on endothelial function are still unknown. Objective: To assess the uptake of NP- and PE-STBEVs by human umbilical vein endothelial cells (HUVECs) and their impact on nitrosative stress, which is associated with endothelial dysfunction. Methodology: All the protocols were approved by the University of Alberta Research Ethics Board. STBEVs were collected using placenta perfusion (pooled from n=3 NP or PE placentas). HUVECs were isolated from umbilical cords of NP. HUVECs were incubated with dyed STBEVs (carboxyfluorescein succinimidyl ester, 80 µg/mL, 1h) to assess STBEV uptake (n=2), and incubated with NP/PE-STBEVs at increasing concentrations (0-200 µg/mL, 24h) to evaluate nitrotyrosine levels (nitrosative stress marker) by immunofluorescence staining (confocal microscopy; n=2). Results: Both NP- and PE-STBEVs were located within the HUVECs. HUVECs nitrotyrosine levels appeared increased with NP-STBEV-concentrations starting at 10µg/mL (n=2:+24% and +23%), while reduced by PE-STBEV (n=2:-17% and -54%). Increasing concentrations from 10-200µg/mL did not further impact nitrotyrosine levels (at 200µg/mL; NP-STBEV:+16% and +49%, PE-STBEV:-19% and -25%). Conclusions: Our preliminary data showed that NP- and PE-STBEVs were internalized by HUVECs and have opposite effects on nitrosative stress, which may suggest NP- and PE-STBEVs differentially impact endothelial function. However, further studies are necessary to confirm our results and to evaluate the effect of PE-STBEVs on endothelial function.
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HQ815TD

Metabolomic analysis by UHPLC-HR-QTOF-MS on ovarian cancer cells with different chemosensitivity.

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Introduction: Ovarian cancer is the fifth leading cause of cancer related deaths in women worldwide, and the second leading gynecological malignancy. Only 15% of patients respond to chemotherapeutics, with 85% of patients presenting with chemoresistance. Accordingly, it is necessary to study the metabolic profile of ovarian cancer cells to understand metabolic differences for the design of new therapeutic targets. Aim. To identify metabolic differences in ovarian cancer cell lines that respond differently to chemotherapy drugs through a metabolomics-based analytical strategy. Methods: Growth Rate 50 (GR50) was determined in SKOV-3 and OVCAR-3 cells exposed to Doxorubicin (DOX), Carboplatin (CP), and Cisplatin (CDDP). For metabolomic analysis, cell extracts were analyzed with UHPLC-HR-QTOF-MS (Compact, Bruker) using a Hydrophobic column (C18) and Hydrophilic column (HILIC), with positive and negative ionization modes. The data were processed and analyzed with Metaboscape Software (Bruker) and Metaboanalyst free online platforms. Results: The analysis obtained with GR50 of DOX, CP and CDDP for SK-OV-3 cells was 0,347 ± 0,199 mM, 17,8 ± 1,3 mM and 606,6 ± 0,77 mM; and for OVCAR-3 cells was 6,7 ± 1 mM, 31,4 ± 6 mM and 535,5 ± 119,5 mM, respectively. The metabolomic analysis showed statistical differences between cells, evidenced by metabolites that characterize this separation. The pathway analysis evidenced differences in amino acid metabolism, CoA catabolism, biotin metabolism, Fatty acid metabolism, and PPP, among the most important ones. Conclusions: The GR50 shows a cytotoxic effect of DOX on SKOV-3 and OVCAR-3, with a higher GR50 value in SKOV-3. CP and CDDP have a cytostatic effect on SK-OV-3 and mild cytotoxic effects on OVCAR-3 cells. We found several metabolic differences between chemoresistant and chemosensitive ovarian cancer cells that could account for its drug resistance observed in vitro, and unveil new therapeutic targets for ovarian cancer treatment.

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DN654LT

Optimizing extracellular vesicles isolation for clinical diagnostic applications.

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Introduction: Extracellular vesicles (EVs) are involved in several intercellular signaling pathways, and transport nucleic acids, lipids, sugars, and proteins. Therefore, EVs have potential as an important clinical tool for the diagnosis and monitoring of several diseases. Although many methodologies have been described for the enrichment of EVs, size Exclusion Chromatography (SEC) has competitive advantages, it does not require special equipment, preserves EVs integrity and biological activity, is reproducible, and is easy to scale up. Objective: To compare and evaluate the isolation efficiency of two in-house packed silico-columns for EVs derived from culture media and human plasma. Methodology: Culture media was recovered from HEK293 cells supernatant. Human plasma was obtained by venipuncture. Samples were centrifugated, ultrafiltrated, and loaded onto packed columns (G200/40 vs G200/120). Profile separation was evaluated by Nanoparticle tracking analysis, Western blot, Flow Cytometry, and gel electrophoresis. Statistical differences between groups were identified by paired t-test. This study was approved by the Human Research Ethics Committees of the University of Concepcion (002643). Results: We found Alix and TSG-101 protein markers between fractions 2 and 7. Optimizing the collection volume improves the ratio between the number of vesicles and protein content (V/P). Fractions 9/10 from G200/40 column offers a higher enrichment of the vesicles (88.3 ± 2.9% vs 82.3 ± 2.7%) with an average size of 85.9 ± 3.6 nm (Mode: 72.8 nm) vs 103.2 ± 10.6 nm (Mode: 93.7 nm) and the lowest V/P ratio with more than 80% of vesicles expressing CD63 and CD81 markers. Conclusion: Using the proposed methodology, we were able to obtain an enrichment of EVs from cell culture media and plasma using both columns, although a much more enriched and homogeneous fraction is achieved with the column G200/40. The methodology is reproducible and easy to implement into routine laboratory practice.

Beca de Doctorado, Dirección de Postgrado, Universidad de Concepción, Proyecto FONDECYT N° 1170809, VRID Asociativo 220.072.043-M.

ML234KT

Experimental and mathematical study of the excitation-contraction coupling in murine skeletal muscle fibers using the fast Ca2+ dye Mag-Fluo-4

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The excitation-contraction coupling (ECC) mechanism in the skeletal muscle links the sarcolemmal electrical phenomena to the mechanical contraction, through the sarcoplasmic Ca2+ handling. Although Mag-Fluo-4 is a fast Ca2+ dye increasingly used for the study of the ECC, its properties have not been well characterized. In the present work we performed fluorescence quenching experiments and isothermal titration calorimetry measurements to characterize the binding thermodynamics of the Ca2+—Mag-Fluo-4 reaction, in order to obtain the stoichiometry, the enthalpy (ΔH) and entropy changes (ΔS), as well as the Kd values in vitro and in situ. The fluorescence maximum (Fmax), minimum (Fmin), and the dye compartmentalization were also determined in muscle fibers obtained from the flexor digitorum brevis of adult mice. An entropically driven reaction, with a stoichiometry of 0.5 for Ca2+/Mag-Fluo-4 was found. Along with the Fmax, Fmin, and an in situ Kd of 636.3 µM, we calculated a peak Ca2+ concentration for the fastest fibers of 14.3 µM. For the first time, a multi-compartment, comprehensive, mathematical model was developed to simulate the Ca2+ movements during single Ca2+ transients in the sarcoplasm, the sarcoplasmic reticulum and the mitochondria, in the continuum of mammalian skeletal muscle fiber types (I. IIA, IIX/D, IIB). The results and implications of these experiments will be discussed during the presentation.
CODI 7858 from 2015

Ca2+ influx inhibition by carvacrol prevents platelet adhesion to endothelial cells under chronic beta-adrenergic stimulation.
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Introduction: The endothelium is a tissue composed by a monolayer of endothelial cells (ECs) that lining the inner face of blood vessels, with an essential role in hemostasia due its fine regulation over coagulation and fibrinolysis. During pathological process including sepsis, ECs become from an anticoagulant and profibrinolytic phenotype to a procoagulant and antifibrinolytic one, in which platelets adhere to ECs and promote microthrombi formation, disseminated intravascular coagulation, multiorgan failure and high mortality rates, that actually represent 19.7% of death causes and are directly related with hemostasia alterations.
Chronic adrenergic activity is observed during sepsis, and has been reported that high levels of circulant catecholamines and the subjacent rise in β-adrenergic receptor (βAR) activity cause deleterious effects on endothelial function, including the reduced expression of anticoagulant and profibrinolytic molecules. In that sense, it has been reported that chronic β-AR activity has adverse effects in some tissues, in part by arising Ca2+ influx. It is known also that Ca2+ permeability regulates some endothelial functions in sepsis, including permeability and traffic of hemostasiarelated molecules. However, the role of Ca2+ conductance in platelet-endothelium adhesion induced by chronic β-AR stimulation has not been elucidated yet.
Aim: To determinate the role of Ca2+ conductance in platelet adhesion to ECs under chronic β-AR stimulation.
Methodology: ECs and platelets from healthy volunteers were co-cultured (n=3) under chronic β-AR stimulation with/without isoproterenol and with/without inhibition of Ca2+ conductance with Carvacrol. Results are presented as mean ± SD. Significant differences were assessed by one-way ANOVA followed by Dunnett's post hoc test. The experimental protocols were approved by the Committee of Bioethics and Biosafety of the Universidad Andres Bello.
Results: Ca2+ conductance inhibition using carvacrol prevents platelet-endothelium adhesion induced by chronic β-AR stimulation
Conclusion. Ca2+ conductance regulates platelet-endothelium adhesion under chronic β-AR stimulation.
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Area: Systems Physiology

TS762LP

Ethanol's disruptive effects on the hypoxic ventilatory response and blood parameters associated with hypercapnia in rat pups

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Introduction: Prenatal and neonatal ethanol (EtOH) exposure affects neonatal respiratory neuroplasticity; a risk factor related with the Sudden Infant Death Syndrome (SIDS). This association is currently promoting research based on the early effects of the drug upon the respiratory system. Objective: The aim of this study was to examine if early acute and/or chronic EtOH exposure are able to trigger hypoxia-related long term facilitation (LTF) associated with elevated pCO2 levels in blood. Methods: At postnatal day (PD) 9, we analyzed the impact of different EtOH doses (0.75, 1.37 or 2.0 g/kg) upon the respiratory response in pups that were chronically pre-exposed to 0.0 or 2.0 g/kg EtOH during PDs 3, 5, 7. At PD 9 animals were subjected to sequential air conditions defined as initial normoxia, hypoxia and recovery normoxia. Furthermore, we analyzed EtOH-related blood parameters in other neonates that were only exposed to 0.0 or 2.0g/kg of EtOH during PDs 3-9 (not subjected to a hypoxic challenge). All experimental protocols were approved by CICUAL-INIMEC. Between-within ANOVAs were performed and data was reported as mean+/- standard error; n>10 in all experimental groups. Results: Sensitization effects upon initial normoxia and the hypoxic ventilatory response in EtOH pre-treated animals were observed. During hypoxic and recovery normoxic events, EtOH pre-exposure increased respiratory rates across all EtOH doses administered at PD9; a phenomenon probably related with a LTF process. Also, at PD9 we observed that all EtOH doses during recovery normoxia elicited LTF. Blood parameters of acidosis-hypercapnia (lower pH and higher pCO2) were observed as a function of either acute or chronic EtOH exposure. Conclusion: Early EtOH exposure induced acidosis-hypercapnia that may support the LTF process observed in hypoxic and recovery normoxic phases. In summary, early acute or chronic EtOH experience alter the acid-base equilibrium inducing respiratory plasticity such as LTF.
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HQ555MQ

Varicocele incidence indicates venous congestion as a potentially pathogenic factor at high altitude.

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Introduction: Chilean miners working at high altitude (HA) often complain of testicular pain. Varicocele (VC), a venous congestion at the pampiniform plexus, constitutes a frequent cause of testicular pain. Venous congestion associated to tissue pressure elevation may represent a prevalent pathogenic pattern at HA. Objectives: To investigate a possible relationship between VC incidence and working at HA. Methodology: 442 males (mean age 38 y) working at mining sites A (< 2,400 m; n=157), B (3,000-3,900 m; n=83) and C (> 3,900 m; n=202) agreed in 2017 to be examined by a physician for the presence of palpable, either visible or not visible VC. Concomitantly, they were subjected to a general medical check and various complementary evaluations. The project was approved by the Ethic Committee for Research in Human Beings, Faculty of Medicine, University of Chile. For statistical analysis prevalence of VC was compared used Chi2 test in descriptive analysis and Logistic Regression was used to evaluate the association between VC and HA. Results: Incidence of VC was significantly (p<0,0001) higher in C (56.7%) than in A (6.3%). A strong association was found between VC incidence and HA: OR=14.6 [95% CI: 5.9 to 36.5] (p≤0,001). Conclusions: VC as a consequence of HA exposure could be clinically important for its own sake. It may represent, moreover, a pathogenic sequence often occurring on exposure to HA. Venous outflow restriction should be further investigated as a potentially crucial pathogenic factor at HA.
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SN918SH

Preliminary analysis of the relationship between periodontal disease and physical performance and muscle injuries in high-performance athletes

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Introduction: Chronic periodontal disease causes elevated blood levels of cytokines, which could play an important role in the source of muscle fatigue during exercise and post exercise oxidative stress, interfering with metabolic processes. Fatigue can cause muscle cramps, making the muscle more susceptible to injuries that persist after other co-variables as anxiety for injuries, and psychophysical stress. Objective: analyze the relationship between periodontal diseases and physical performance and incidence of muscle injuries in professional male soccer players. Methodology: Project was approved by the Committee of Bioethics of the School of Odontology of the National University of Rosario including informed assent. Twenty professional male soccer players aged 18 – 22 year-old from Club Newell's Old Boys Rosario were evaluated. Dental evaluations were carried out by Green Vermillion index (GVI) and periodontogram. In addition, strength test (squats) and Intermittent Fitness Test (IFT) were analyzed. Then, information on muscle injuries along the season was collected by self-report and medical report. The statistics was carried out with the software SPSS by t student method (mean±SD). Results: The results showed that GVI was 0-1 in eight players (group 1), 2 in eight players (group 2) and 3 in four players (group 3). 5 players of group 1 showed muscle overload (0.625±0.517; n=8) vs 8 players of group 2 (1±0; n=8) ($p=0.05$). While 1 player of group 1 suffered cramps (0.125±0.353; n=8) vs 6 players of group 2 (0.75±0.462; n=8) ($p<0.05$; $p=0.0053$). GVI showed a statistically significant correlation for all muscle injuries ($p<0.05$; $p=0.0213$) between group 1 and 2; but in the case of the squats or IFT, no statistically significant correlations were observed. Conclusion: these findings suggest that the chronic systemic inflammation of low grade that arises from the oral illnesses is a factor of potential risk for sports injuries and relapses of injuries.

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CJ815TT

High fat diet and natriuretic peptide system in skeletal muscle

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Introduction: It has been postulated that the combination of an increased fat mass and a decreased skeletal muscle (SM) mass favors the development of cardiovascular diseases. Natriuretic peptides (NP) modulate cardiovascular homeostasis but the knowledge about their role on SM is limited. The bioavailability of NP depends on the neprilysin enzyme (NEP) that is responsible for the degradation of NP, and the natriuretic peptide receptor C (NPR-C) that acts as a clearance receptor. Objective: To evaluate the expression of components responsible for the degradation of NP in SM of rats fed high-fat diet (HFD) for 11 weeks. Experimental design: Wistar rats received, from weaning until the 14th week of life, HFD (60% of fat) or standard diet (SD). At the end of the experimental period, body weight (BW) and systolic blood pressure (SBP) were measured, and oral glucose tolerance test (OGTT) was performed. Animals were sacrificed, and NEP and NPR-C expression were evaluated (RT-qPCR) in SM. The protocol was approved by Ethic Committee CICUAL-FFyB-UBA. Results are expressed as mean±SEM. Statistical analysis: Student test (n=6 rats/group; *$p<0.05$; **$p<0.01$; vs SD). Results: BW was increased in HFD rats, but SM was similar between both groups (BW(g): SD=476.5±10.9, HFD=542.6±11.1**; SM/tibial length(g/cm): SD=48.8±1.4; HFD=46.4±2.2). Also, SBP (mmHg) was similar between groups (SD=126±4; HFD=131±3). Both area under the curve (AUC) and glycemia at 180 minutes from OGTT were greater in HFD rats, showing alterations in carbohydrate metabolism in this group (AUC(min.mg/dL): SD=24200±1151, HFD=28310±959*; Glycemia(mg/dL): SD=114.4±5.8; HFD=147.8±7.0**). NEP and NPR-C were increased in HFD group (NEP/GAPDH: SD=0.58±0.06; HFD=0.96±0.08**; NPR-C/GAPDH: SD=0.59±0.05; HFD=0.86±0.07**). Conclusion: HFD induced the increase of both components of NP system involved in the degradation of NP. Therefore, HFD would reduce the bioavailability of NP in SM, and this finding could be involved in the development and/or maintenance of cardiovascular diseases linked to obesity.

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QN872KN

Influence of atrial conduction (local theory) on the origin of the P-wave dispersion phenomenon

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FD971HM

Role of the vectorial theory in the occurrence of the P-wave dispersion phenomenon

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JF324BG

Impact of aerobic training on the metabolism of the adipose tissue of spontaneously hypertensive rats

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Introduction: cardiovascular diseases are the first cause of death in Argentina and worldwide, being hypertension the main modifiable risk factor. There is an association between hypertension, insulin resistance (IR), and oxidative stress (OS). Interestingly, physical training delays the progression of cardiovascular disease and improves insulin sensitivity, possibly involving modifications in adipose tissue (AT). Objective: To determine the effects of aerobic training (Tr) on IR, OS, and AT phenotype in spontaneously hypertensive rats (SHR). Methodology: SHR were randomly assigned to a sedentary group (S) or subjected to a swimming routine for 8 weeks (Ex). OS was determined by TBARS and O2- production measured by the lucigenin method in AT. To study the insulin response, Glut4 expression and phosphorylation were measured in adipocyte membrane fractions and a glucose tolerance curve (GTC) was performed. Changes in the AT phenotype were analyzed in histological sections by light microscopy. Results are expressed as mean±SE (n) and compared by T-test, considering $p \leq 0.05$ as statistically significant. The animal protocols were approved by the Care and Use of Laboratory Animals of our institution. Results: Tr decreased O2- production [23.12 ± 11.27% S(9) vs. Ex(8), $p<0.05$], effect accompanied by a downward trend in TBARS [S(8) vs Ex(6)]. On the other hand, exercise training also increased the insulin-induced translocation of pGlut4 [46.29 ± 21.44% (6)], while no effect was observed in S. This effect correlated with a decrease in the area under the GTC [29.59 ± 9.58 (4) compared to group S(3), $p<0.05$]. Finally, a transition from unilocular to multilocular was observed in the AT of Ex, although the results did not reach statistical significance so far [S(4) vs Ex (4)]. Conclusion: Tr emerges as a potentially effective strategy to improve AT phenotype and function with positive metabolic consequences in a murine model of hypertension and cardiovascular disease.

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RT325TD

Ethanol's effects upon neonatal breathing plasticity and ethanol affinity as a function of late prenatal exposure to ethanol moderate doses.

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Introduction: Since fetal alcohol exposure has been described as a risk factor of Sudden Infant Death Syndrome, preclinical research has focused on the impact of drug exposure upon respiratory plasticity. Fetal and neonatal respiratory alterations have been described in different studies. Via associative learning processes, fetuses also acquire affinity towards the drug. Aim: The overall aim of the study was to analyze whether prenatal memories involving ethanol and/or acetaldehyde are reactivated during postnatal life with an impact upon respiratory plasticity and ethanol affinity. Methodology: During gestational days 17-20 dams received a subcutaneous injection of D-penicillamine (50 mg/kg) or saline (0.9% NaCl). After 30 minutes, rats received an intragastric administration of ethanol (2.0 g/kg) or water. At postnatal days 4 and 6, a total of 259 pups were evaluated in an artificial lactation test while simultaneously recording their respiratory patterns. Four groups were defined: M-M, M-E, E-M or E-E (M or E represent milk or ethanol intraoral infusions during each day). All procedures were certified by the Institutional Animal Care and Use Committee, complied with the ARRIVE guidelines and the Guide for the Care and Use of Laboratory Animals. Between-within ANOVAs and Duncan tests were used to analyze the data. Results: Brief prenatal ethanol experience generated depressant respiratory effects and increased apneic disruptions. There was also evidence indicating that ethanol consumption was significantly higher in pups prenatally exposed to ethanol. Sequestering acetaldehyde via prenatal D-penicillamine inhibited this effect. Conclusion: Neonatal respiratory plasticity is affected by moderate prenatal ethanol exposure. In terms of ethanol affinity, the results indicate that intrauterine acetaldehyde is crucial when considering later ethanol affinity.

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KQ867HF

Moderate aerobic exercise training prevents the augmented hepatic glucocorticoid response induced by high-fat diet in mice

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Introduction: Glucocorticoids (GCs) are critical regulators of energy balance. Their deregulation is associated with the development of obesity and metabolic syndrome. However, it is not understood if obesity alters the tissue glucocorticoid receptor (GR) response, and moreover whether a moderate aerobic exercise prevents the alteration in GR response induced by obesity. Objective: Investigate the consequences of HFD-induced obesity in the GR sensitivity in relevant tissues that control energetic homeostasis and the effects of moderate aerobic training in this response. Methods: To evaluate the GR response in obese mice, we fed C57BL6J mice with a high-fat diet (HFD, n=6) or control diet (CD, n=6) for 12 weeks. Before mice were sacrificed, we injected them with dexamethasone. To assess the exercise role in GR response, we fed mice an HFD (n=6) or CD (n=6) and subjected them to moderate aerobic exercise three times a week. All results are represented as the mean ± SEM. Student's t-test of independent samples analyzed the results for the comparisons of two groups. For comparisons greater than two groups, we used a two-way ANOVA test followed by Tukey's post-test. Animal care and procedures were approved by the Animal Ethics Committee of the University of Chile. Results: We found that mice fed a high-fat diet for 12 weeks developed hepatic GC hypersensitivity without changes in the gastrocnemius or epididymal fat GR response. Therefore, moderate aerobic exercise improved glucose tolerance, increased the corticosterone plasma levels, and prevented hepatic GR hypersensitivity with an increase in epididymal fat GR response. Conclusion: Collectively, our results suggest that mice with HFD-induced obesity develop hepatic GR sensitivity, which could enhance the metabolic effects of HFD in the liver. Moreover, exercise was found to be a feasible non-pharmacological strategy to prevent the deregulation of GR response in obesity.
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DL856JH
Dietary reversion of protein malnutrition induces type 2 diabetes in adult rats.
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Introduction: Growth restriction in utero is associated with the development of obesity and diabetes. The current understanding is that intrauterine deprivation programs the individual for a deprived environment, and that such programming is maladaptive in a no deprived environment. The liver plays an essential role in metabolism regulation. The aim of this study was to evaluate the effects of protein malnutrition during development stages followed by refeeding with normal diet on glucose metabolism in adult rats. Methodology: Pregnant rats received a Low Protein Diet (LPD 8%-protein) during gestation and lactation. After weaning the offspring received LPD until 60 days, after that the diet was changed by a Control Diet (CD 20%-Protein) until 120 days old (Reverted Group =RG). Control Group (CG) received only a CD.(All Experiments were approved by our Institutional Animal Care Committee FCEyN-UNMDP RD 140/15.). Comparison between groups were analyzed by using non-parametric unpaired two-tailed Student's t test. All data are expressed as mean±SEM. Values of $P< 0.05$ were considered statistically significant. Total n=6 for each experimental groupResultsProtein malnutrition during gestation, lactation and childhood, followed by normal refeeding further increased serum glucose and AUC during ivGTT ($p<0.05$). Triglyceride, cholesterol, HOMA, insulin, leptin, adiponectin values were significantly higher in the RG group than CG ($p<0.05$). These changes were associated with increased expression of hepatic lipogenesis enzymes ACC (acetil-CoA carboxilase) and FAS (Fatty acid synthase) ($p<0.05$), glucose transporter (GLUT2; $p<0.05$), and insulin signaling protein (IRS2, $p<0.05$), and decreased glycogen synthase expression (GS, $p <0.05$) in the liver. Confirmed by PAS diminished staining in RG. Urinary excretion of C-peptide (co-secreted insulin) in RG group was greater than CG ($p<0.05$).Conclusion: Reverting to control diet after a severe protein malnutrition throughout the development stages increased liver de novo lipogenesis. This has a fundamental role in type 2 diabetes pathogenesis.
CONICET-UNMDP

QG247RM
Metabolic flexibility in metabolically healthy vs. metabolically unhealthy non-obese subjects
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Introduction: Metabolic flexibility (MetFlex) is the capacity to adapt fuel oxidation to fuel availability. An impaired MetFlex is postulated as an early stage driving lipotoxicity in skeletal muscle, thus disturbing metabolic health even in non-obese subjects. Objective: To compare MetFlex between metabolically healthy and unhealthy non-obese subjects. Methodology: Subjects were considered as unhealthy if they were afflicted with the metabolic syndrome, defined as having ≥3 of the following factors: elevated glucose, elevated triglycerides, elevated blood pressure, elevated waist circumference, and reduced HDL-cholesterol. Subjects were considered as healthy if having <3 of those factors. MetFlex to glucose was measured during an euglycemic-hyperinsulinemic clamp (85 mg/dL glucose, 1 mU insulin/kgxmin), and MetFlex to lipid during an exercise bout (50% VO2max, 2 h). MetFlex was operationalized as the change (delta) in respiratory quotient during the clamp (RQinsuln–RQfast) and during exercise (RQ2h–RQmax). The Ethics Committee at the Pontificia Universidad Católica de Chile approved all procedures (no.180508004). Results: Healthy (2 males, 3 females) vs. unhealthy (1 male, 3 females) subjects had similar age (mean [SD]; 53.1 [4.6] vs. 50.4 [5.4] years), BMI (23.5 [3.1] vs. 24.8 [1.2] kg/m2), fat mass (31 [13] vs. 34 [6] %), insulin sensitivity (8.6 [3.7] vs. 7.1 [2.6] mg/kgxmin), and VO2max (1.9 [0.7] vs. 1.5 [0.7] L/min). RQ increased during the clamp (P=0.005 insulin-effect) and trended to decrease during exercise (P=0.089 exercise-effect), without differences between groups (P>0.40 for insulin-group or exercise-group interactions; two-way ANOVA). Consequently, healthy vs. unhealthy subjects had a similar deltaRQ during the clamp (0.06 [0.03] vs. 0.04 [0.05], P=0.44; t-test) and exercise (–0.06 [0.06] vs. –0.04 [0.03], P=0.58; t-test). Conclusion: MetFlex does not appear impaired in metabolically unhealthy non-obese subjects. Other factors may thus be involved in the development of metabolic syndrome. These preliminary findings need to be confirmed with a larger sample size. Fondecyt de Iniciación #1118036

RS734BJ

Effect of TLR4 in the oxidative stress induced by a murine model of renal ischemia and reperfusion: Potential role of NOX-2

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Introduction: Renal ischemia and reperfusion (I/R) cause acute kidney damage and Toll-like membrane receptors (TLRs) activation. The TLR4 receptor plays a critical role in inflammation during I/R. The phagocyte NADPH oxidase (NOX2) is involved in oxidative stress and limits inflammation by modulation pathways that affect neutrophil accumulation and clearance. Objective: To determine the role of TLR4 during the renal I/R in the upregulation of markers of oxidative stress in Wild type (Wt) and Knockout (KO) animals for TLR4. Methodology: C57BL/6 Wt and KO mice were subjected to 30min of renal ischemia and 48h of reperfusion. The kidney damage was evaluated by NGAL, histology (H/E and PAS stained), oxidative stress by lipoperoxidation and NOX-2. Procedures were approved by the Committee on the Ethics of Animal Experiments of the University de los Andes, Chile. Data were analyzed with non-parametric Kruskal-Wallis ANOVA, Mann-Whitney U test or post-hoc Tukey test. Significance was set at $p<0.05$. Data are presented as the mean ± standard error. Results. The histological analysis showed that both groups (Wt and KO) showed tubular damage (loss of tubular structure and decreased brush border) induced by I/R. NGAL was increased by I/R in the cortex and medulla of Wt and KO animals. The NOX-2 expression was observed in the cortex and medulla form controls kidney of WT and KO animals. The renal I/R upregulated the NOX-2 protein only in the medulla of KO animals. The lipoperoxidation levels increased in the same levels by I/R in Wt and KO animals. Conclusions: The absence of TLR4 during the I/R renal did not significantly prevent kidney injury (NGAL), tubular damage, and oxidative stress. The induction of NOX-2 by I/R was observed only in the medulla of KO animals, suggesting that this protein is probably involved in the renal damage observed in TLR4-KO animals.
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CP864JD

Effect of training guided by heart rate variability versus predefined training on maximal aerobic power or speed: a systematic review with meta-analysis.

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Introduction: The training load affect the regulation of the autonomic nervous system (Yanlin, Fei, & Shengjia, 2020), in this way the variability of the heart rate (HRV) is a useful marker of load internal (Bourdon et al., 2017) and monitoring responses and adaptations to training (Roos et al., 2013). This physiological basis has meant that HRV has been used to guide training with similar results to predefined training on maximum aerobic power or speed (WMax) (Javaloyes et at., 2019a; Javaloyes et al., 2019b; Kiviniemi et al., 2010; Nuuttila et al., 2017; Da Silva et al., 2019). Objective: To perform a systematic review and meta-analysis to determine if HRV-guided training (HRV-g), compared to predefined training (PT), maximizes the further improves on WMax in healthy individuals. Methodology: A systematic search of PubMed, Web of Science, and the Cochrane Library databases (up to August 2020) was performed. This analysis included randomized controlled trials assessing the effects of HRV-g on WMax in subjects untrained, physically active, and well trained. The methodological process was based on the recommendations indicated by the PRISMA

(preferred reporting items for systematic review and meta-analysis) statement. 6 articles (141 participants) qualified for inclusion. A random effects model was applied to determine the effect of HRV-g on WMax. The effects of training on this outcome between HRV-g and PT groups were expressed as standard mean differences (SMD) and their 95% confidence intervals (CI). Results: HRV-g and PT led to a significant increase in WMax (HRV-g: SMD= 0.66, 95% CI 0.33, 0.98; p<0.0001; PT: SMD= 0.48, 95% CI 0.12, 0.83; p=0.009). However, HRV-g did not show significant differences in WMax (SMD= 0.06, CI -0.26, 0.38; p=0.72). Conclusion: We conclude that HRV-g periodization is an effective method for improving WMax. However, so far there is no evidence that it is more effective than PT.

TM222NP

P2Y2 mRNA in smooth muscle of small intrapulmonary vessels obtained by laser microdissection from Precision Cut Lung Slices

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Introduction: Purinergic signalling participates in pulmonary vascular physiology and pathophysiology with P2Y2 receptor mediating arterial smooth muscle cell (SMC) contraction. In small intrapulmonary veins, P2Y2 participation in ATP induced vasoconstriction is reported. Due to airway branching, reduction in diameter and regional diversity causing limitations in small intrapulmonary vessels study, the Precision Cut Lung Slices (PCLS) method emerged. However, PCLS is not enough to isolate SMC to study P2Y2 expression. Combination of PCLS with laser microdissection, not previously reported, could facilitate studying P2Y2 in SMC of intrapulmonary vessels. Aim: To determine the presence of P2Y2 mRNA in SMC of intrapulmonary vessels obtained by laser microdissection from rat PCLS. Methodology: PCLS from healthy rat were obtained as previously described (Henriquez et al. The Journal of Physiology 2018;596(13):2491–506), dehydrated and fixed with acetone. The SMC from small intrapulmonary vessels were isolated using laser microdissector (Leica). The SMCs were homogenized in Trizol and total RNA integrity was determined in agarose gel. qRT-PCR with 0.4-0.5 ug of total RNA was performed using primers to P2Y2, and control primers to alpha-smooth muscle actin (alpha-SMA) and 18s. Brain, aorta and microdissected parenchyma were used as control. Cycle threshold (Ct) is shown (preliminary data, n = 1 per sample). CBA1044 protocol was approved by the Institutional Animals Welfare Bioethics Committee, University of Chile. Results: 43 SMC of small intrapulmonary arteries and veins and 176 parenchymal regions were microdissected. Total RNA of vascular SMC showed slight degradation. Ct in samples was 30.875 for P2Y2 in SMC, 22.625 for P2Y2 in brain, 19.27 for alpha-SMA in SMC, 11.67 for alpha-SMA in the aorta, and 19.66 for 18s in the parenchyma. Conclusions: P2Y2 mRNA expression in small intrapulmonary vessels was demonstrated by laser microdissection from PCLS. The combination of both techniques will facilitate molecular studies in distal structures in the lungs. Financed by URG-035/18

NK869DR

Comparison between echocardiography and cardiorespiratory capacity in a pulmonary arterial hypertension rat model.

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Introduction: Pulmonary arterial hypertension (PAH) is a vasculopathy life-threatening condition, characterized by progressive elevation in mean pulmonary artery pressure (mPAP) ⩾25 mmHg at rest, measured by right heart catheterization. The echocardiography is a non-invasive method that allows to estimate the mPAP in PAH animal models. However, it is not clear the advantages front other important test like the cardiorespiratory capacity that it decreases as a function of progression of PAH. Objective: To characterize the progression of echocardiographic variables and the cardiorespiratory capacity in a PAH animal model. Methods: Eight-week old male Sprague Dawley rats (~350 g) were used according to our protocol approved by Institutional Animal Care and Use Committee. PAH was induced using subcutaneous injection of 60 mg/kg of monocrotaline (HAP group; n=5), vehicle was injected to the control group of rats (CG; n=5).Cardiorespiratory fitness was measured weekly in treadmill with an incremental test. Echocardiography was performed under general anesthesia. Transthoracic echocardiography was performed weekly by Vivid i (General Electric, USA) ultrasound device and the iRL-RS probe, 5-13 MHz. Statistical difference between the parameters at baseline and after PAH induction was assessed with Kruskal-Wallis. Mann-Whitney analysis for intergroup and Friedman test for repeated-measures was used. P-value less than 0,05 was considered significant. Results: There was a significant decrease cardiorespiratory capacity of the PAH rats in comparison with CG at fourth week. The echocardiographic left ventricle measurements, mass, systolic and diastolic function had not statistical difference between CG and PAH rats, but Pulmonary Artery flow measurements had significant decrease HAP rats in comparison with CG and at fourth week. The Pulmonary Artery Acceleration Time (PAAT) started to decrease at third week after PAH induction (p= 0,0204). Conclusions: The echocardiographic variable Pulmonary Artery Acceleration Time was more sensitive than cardiorespiratory capacity for detecting the progression of PAH in rats.
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RD311ND

The Impact of the Potential Antitumor Agent 2-(4-hydroxyphenyl) Amino-1,4-naphthoquinone (Q7) on Vasomotion is Mediated by the Vascular Endothelium, but not Vascular Smooth Muscle Cell Metabolism

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Introduction: Vasomotion is defined as rhythmic oscillations in arterial diameter, which regulates the blood flow and the blood pressure. Objective: Since antitumor treatment may impair vascular functions and increase the blood pressure, we sought to evaluate whether a new naphthoquinone derivative used as antitumor agent causes adverse effects on vascular function. Methods: We evaluated the toxicity of 2-(4-hydroxyphenyl) amino-1,4-naphthoquinone (Q7) and its effects on vascular vasomotion in three models of vascular structure; endothelial cells, aortic ring and smooth muscle cells. ANOVA one-way was used and post-hoc test Bonferroni. Data represents the standard error of the mean of 3-4 independent experiments. *p<0.05 versus Control. The trials were approved by the Ethics Committee of Arturo Prat University (CEC-17). Results: Q7 did not demonstrate any toxic effects, yet inhibited the formation of tubular capillary-like structures of the EA.hy926 hybrid endothelial cell line grown on Matrigel. In ex-vivo experiments with aortic rings pre-contracted with phenylephrine (PE, 10-6 M), Q7 (10-5 M) significantly (p<0.05) reduced vascular rhythmic contractions induced by the acetylcholine (ACh; 10-7-10-5 M), while sodium nitroprusside (a nitric oxide donor; 10-8 M) recovered the vasomotion. Q7 (10-5 M) neither decreased the KCl-induced vascular rhythmic contractions in aortic rings pre-contracted with BaCl2 (a non-selective blocker K+ channels; 10-3 M). Glucose uptake in vascular smooth muscle cells (A7r5) pre-incubated with Q7 (10-5 M) for 3 h was also decreased, but not ATP content, suggesting that the rapid reduction in vasomotion observed in vascular reactivity experiments does not involve cellular metabolism, but could be due to faster mechanisms involving endothelial nitric oxide (NO) and K+ channels leading to oscillations in intracellular Ca2+. Conclusions: Vasomotion contributes to the evaluation of the vascular function in presence of a new naphthoquinone derivative with low cytotoxicity.

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MN547QF

NFAT5 and AQP-1: a new molecular mechanism involved in renal ischemic preconditioning is modulated by aminoguanidine.

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Introduction: NFAT5 is a protective factor during renal ischemia and reperfusion (IR). Ischemic preconditioning before IR (IPC-IR), ameliorates renal injury. Moreover, iNOS is upregulated in IR and IPC-IR. There is no information about NFAT5 during IPC-IR and the iNOS effect. Aim: The aim of this study was to investigate the effect of IPC-IR on NFAT5 and AQP-1 expression and the participation of the inducible nitric oxide synthase (iNOS). Methods. Mice were subjected to sham, IR (IR:30min/30min) or IPC-IR (two cycles of IR:5min/5min, then IR:30min/30min). Additionally, we studied the effect of aminoguanidine, an iNOS inhibitor, on the IPC-IR protocol (AG-IPC-IR). The effect of AG was also evaluated in cells exposed to hypoxia. Procedures were approved by the Committee on the Ethics of Animal Experiments of the University de los Andes, Chile. Data were analyzed using the non-parametric Kruskal-Wallis ANOVA and Mann-Whitney U test or post-hoc Tukey test. The level of significance was set at p<0.05 Data are presented as the mean±standard error. Results: We found that IPC-IR: 1. Increased NFAT5, AQP-1 and INOS mRNAs compared with sham. 2. Immunohistochemistry showed increased NFAT5 nuclear signal, AQP-1 luminal abundance and INOS in tubular cells. The AG-treatment before IPCIR: 1. downregulated NFAT5, AQP-1 and INOS mRNA 2. Decreased nuclear and luminal signal for NFAT5 and AQP-1, respectively. 3. Improved the kidney tubular morphology. 4. Interestingly, AG upregulated the NFAT5/AQP-1 pathway by hypoxia in cell culture. 5. Finally, AG-IPC IR prevented the loss of glomerular filtration rate; Glutation-S transferase activity and lipoperoxidation (TBARS). Conclusions: Taking together, our results provided a relevant and novel evidence of

molecular bases of kidney protection against ischemia and reperfusion injury. AG-IPC-IR protocol is a good opportunity to explore potential therapeutic tool to prevent deleterious effects of renal IR.This is particularly important in kidney preservation, as for example occurs during renal transplant
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JS916CQ

Quercetin induced white adipose tissue browning and attenuate high-fat diet-induced glucose intolerance: implications of the FNDC5/irisin pathway in muscle and in L6 myotubes.

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Introduction: Irisin is an exercise-induced myokine that can induce browning of white adipose tissue (WAT), through upregulation of the uncoupling protein-1 (UCP-1), and other metabolic benefits. We previously observed that supplementation with the flavonoid quercetin (Que) mitigated high-fat diet (HFD)-induced glucose intolerance and adipose hypertrophy. Aim: To evaluate whether these beneficial effects could be related to Que capacity to activate muscle FNDC5/irisin and UCP-1 and brown markers in the subcutaneous WAT (SAT) of rats consuming a HFD. Also, the role of Que on FNDC5/irisin pathway in L6 myotubes triggered with palmitate. Methods: Rats (n=21) were divided in 3 groups: Control (Ctrl), HFD, and HFD supplemented with Que (20 mg/kg body weight/d) for 6-weeks, (protocol approval N° 36/2014). L6 myotubes were treated with/without Que (1uM) and subsequently with/without palmitate (0.5 mM). Statistical analysis: Data are shown as mean ± S.E.M. Statistical significances were assessed by one-way ANOVA followed by Bonferroni's Test. A $P<0.05$ was considered statistically significant. Results: Que supplementation significantly increased PGC-1α, FNDC5 and p-AMPK in skeletal muscle compared to Ctrl and HFD groups. In addition, Que significantly upregulated proteins involved in WAT browning (PRDM16, PGC-1α, PPARɣ) and UCP-1 in SAT compared with Ctrl and HFD groups. Moreover, Que partial and significantly increased brown adipose tissue weight compared to HFD and Ctrl groups, respectively. In L6 myotubes Que prevented palmitate-decrease GLUT4, PGC1-α and FNDC5 expression and irisin secretion and also prevented palmitate-downregulated mRNA levels of PGC-1α and FNDC5. In addition, PGC-1α siRNA transfection in L6 myotubes abrogated the effects of Que on FNDC5 expression. Conclusions: Overall, Que enhanced FNDC5/irisin pathway in muscle and L6 myotubes and enhances the expression of transcriptional regulators of browning and UCP-1 in the SAT of rats fed a HFD. These findings support the potential relevance of consuming Que-rich foods to attenuate high-fat diet-induced metabolic dysfunction.
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KR772CJ

Antiarrhythmic action of chronic melatonin in female rats

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Introduction: Arrhythmias are complications during reperfusion of the ischemic myocardium. Acute melatonin is antiarrhythmic, but there is less information about females' animals receiving chronic treatment. Objective: We aim to evaluate the electrophysiological effects of melatonin in female rats. Methodology: Wistar Kyoto rats (WKY), ten weeks old, received melatonin dissolved in water (3-5 mg/kg/day, MEL) during 15 days. After this period, we evaluated the electrocardiogram in isolated hearts undergoing a 10 minutes regional ischemia, followed by reperfusion of 10 minutes. All procedures were approved by local Institutional Animal Care and Use Committees (106/2017). Results: Melatonin treatment shortened PR and QRS intervals and prolonged the QT interval and QT corrected for heart rate. Melatonin chronic treatment prevented the incidence of ventricular fibrillation during reperfusion (WKY 6/9; WKY+MEL 1/10; P=0.0198 by Fisher's exact test). Conclusion: Our results confirmed the cardioprotective effects of oral melatonin administration in female rats and showed electrocardiographic modifications compatible with increased conduction and delayed myocardial repolarization as possible antiarrhythmic mechanisms.
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CP293FN

Impact of female sex hormones on autonomic nervous activity and in heart rate variability.

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Introduction: Heart rate variability (HRV) is understood as the variation in milliseconds between one heartbeat and another. This is influenced by the autonomic nervous system and the different conditions that determine an increase or decrease in the activity of the sympathetic or parasympathetic nervous system. This study aims to analyze the behavior of the variability of the heart rate, through the domains of time, frequency, and nonlinear analysis, and establish the possible influence of female hormonal stimulation taking into account the phase of the menstrual cycle. Methodology: In this study, a sample of 27 women volunteers who met the inclusion criteria were collected (age between 18 and 25 years old, regular menstrual cycle and not using oral contraceptives). An electrocardiogram (EKG) was taken using DII derivation for five minutes at two different times in the cycle: 1) menstrual phase, taken as the first day of menstruation, or the two days before it, and 2) ovulatory phase understood as day 14 ± 2. It should be clarified, that the evaluation of HRV has a very low risk and this protocol was approved by the ethics committee of the Faculty of Medicine. Outcomes: As a result, during the menstrual phase the approximated entropy (ApEn) was 1.10 +/- 0.08 in contrast with the ovulation phase that was 1.15 +/-0.08 with a statistical difference (p = 0.004) which suggests less activity of the parasympathetic nervous system during the menstrual phase. Conclusion: It was concluded that there is a relationship between the hormonal stimulus and the activity of the autonomic nervous system, being the most sensitive method for the evaluation of the activity of the approximated entropy. This study demonstrates once again the relationship between the regulatory systems (endocrine and autonomic nervous system).
Thanks to the biomedical physiology laboratory on campus at the University of La Sabana.

GK331LM

Evaluation of the pregnant women rectus abdominis muscle function –An ex vivo contractility analysis using myography

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Introduction: Rectus abdominis muscle (RAM) is responsible for increasing intra-abdominal pressure during the second stage of labor and its voluntary contraction is associated with pelvic floor muscle (PFM) activity. Dysfunction of the PFM can result in urinary and fecal incontinence. There are no previous studies available on pregnant women RAM contractility using Myography. Objectives: The objective of this study was to evaluate the ex vivo RAM contractility of pregnant women using Myography, an ex vivo sensitive technique that evaluates muscle fiber function through controlled electrical stimulation. Methodology: The study group included five pregnant women and for the reference group, elastic tape (inert material) was used. The RAM (1 cm2) was collected during C-section at Perinatal Diabetes Research Center, Botucatu-UNESP Hospital, Brazil. Electrical activity was recorded using DMT820M Multi-Chamber Myograph (ADInstruments, USA) and the LabChart 8 software was used to capture and store the muscle responses. This research was approved by the Institutional Review Board of Botucatu Medical School-UNESP (CAAE: 20639813.0.0000.5411). Statistical method: Kruskal Wallis significance, $p < 0.05$. Data are represented as mean±SD. Results: The clinical characteristics of the pregnant women such as body mass index (36.1±5.31 kg/cm2), maternal age (26.0±6.44 years), gestational age (39.3±1.97 weeks) and maternal blood glucose (81.0±3.00 mg/dL) were determined. Newborns weight (3.02±4.5 kg) and height (47.0±3.08 cm) were recorded. The weight (0.08±0.03 g), length (0.09±0.02 cm) and RAM cross-sectional area (0.09±0.02 cm^2) of the RAM fragments were determined. The average RAM force peak was found to be 7.44 mN. Three of the RAM fragments demonstrated ex vivo electrical activity. Conclusions: This study confirms that myography is a useful technique to analyze the contractility of RAM and complements the clinical practice in the knowledge of muscle physiology. Our preliminary data will serve as the basis for future studies, especially related to the use of this technique for specific purposes.
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RP448QG

High-intensity intermittent games effects on heart autonomic regulation of children 6 to 9 years old with obesity and overweight: a protocol trial

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Introduction: The children obesity is it problem of health on world, there are 41 million of children's overweight or obesity (1). This is the protocol of a study that aims to analyze the effects of an intervention of high-intensity intermittent games on heart autonomic regulation for children have a pattern very similar to High intensity interval training (HIIT), are high intensity intervals alternating for

short periods of rest intervals (2). In children and adolescents, HIIT training strategies have been tested with excellent results, related to both body weight and cardiorespiratory condition, however, the role in the improvement of autonomic control is not clear (3). The hypothesis of this study is that the practice of children's games for age, improve cardiac autonomic regulation by increasing HF and decrease the LH/HF ratio of children aged 6 to 9 years compared to the usual recommendations of medium intensity activity of the control group, (4). Objectives: To evaluate the effect of children's games on cardiac autonomic regulation in children aged 6 to 9 years with Obesity and Overweight. Methodology: Randomized controlled field trial in children aged 6 to 9 years with overweight and obesity for 20 weeks in schools in the city of Neiva, Huila. Physiological variables shall be measured in an experimental group and compared against the control group before intervention protocols. Expectation: At the end of the study, HIIG students are expected to increasecardiac autonomic regulation and improve body composition, also to evaluate the impact of this recreational training time for age, and determine metabolic variables that will also be measured to generate correlations. Conclusions: it is important to establish the effectiveness of intervention programs in physical activity and nutritional education without pharmacological intervention in children and the autonomic analysis can be very useful for this purpose.
Universidad Surcolombiana

FS254NC

Impaired endothelium-dependent vascular function in female mice with a history of a pregnancy complicated by dyslipidemia

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Introduction: Dyslipidemia during pregnancy increases the risk of cardiovascular complications later in life. High circulating levels of oxidized low-density-lipoproteins (oxLDL) are associated with vascular dysfunction via oxidative stress. Whether vascular dysfunction in pregnancies complicated by dyslipidemia persists postpartum is unclear. Objective: To evaluate whether dyslipidemia-induced vascular dysfunction in pregnancy impairs maternal long-term vascular function. Methods: Pregnant C57BL/6 mice were fed a high-cholesterol diet (HCD) between gestational day 13.5 and term. Control pregnant mice were fed a standard chow diet (CD). Non-pregnant females were on the same diets for an equal period. At 3 months postpartum (or 3 months post-diet), aortas were isolated to assess ex-vivo vascular function by wire myography (n=3-7). Vascular responses to methacholine (MCh) were evaluated in the presence or absence of oxLDL (50 µg/mL) or L-NAME (100 µM). Superoxide levels were evaluated in aortic sections by dihydroethidium staining. Data (mean±SEM) were analyzed by two-way ANOVA with Sidak's posthoc analysis. Ethics committees approved animal experiments following the Canadian Council on Animal Care Guidelines. Results: In the postpartum females, the HCD reduced maximal MCh-induced vasodilation versus CD group (65.3±19.0 vs 88.5±7.2%; p=0.02), while no effects of HCD were found in non-pregnant mice. HCD reduced nitric oxide contribution to vasodilation in the postpartum (HCD:183.8±10.5 vs CD:256.5±13.4 AU; p=0.001) and non-pregnant females (HCD:135.4±15.2 vs CD:252.1±19.8 AU; p=0.04). Pre-incubation with oxLDL reduced maximal vasodilation only in postpartum HCD females versus control aortas (33.5±1.0 vs 65.3±19.0%; p=0.004). HCD tended to increase aortic superoxide levels in postpartum females (HCD:4.5±2.1 vs CD:3.6±1.1 AU) while decreasing them in non-pregnant females (HCD:5.5±1.2 vs CD:3.6±0.6 AU; interaction p=0.06). Conclusion: A high-cholesterol diet during pregnancy impairs maternal long-term vascular function, potentially via increased oxidative stress and lower nitric oxide contribution. Our study suggests that vascular dysfunction during gestational dyslipidemia persists after pregnancy, which could contribute to long-term cardiovascular complications.
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FP575DG

Corticosterone and ACTH secretion is recovered after immune challenge or acute immobilization in sepsis survivor animals

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Introduction: Clinical and experimental studies report a dysregulation of hypothalamic-pituitary adrenal axis during sepsis causing hormone secretion alterations contributing for the pathophysiology of the disease. However, it is unclear whether theses alterations persist even after the disease remission. Objective: We evaluated the effect of an immune challenge or restraint stress on the hormones secretion of HPA axis in sepsis survivor animals. Material and Methods: Sepsis was induced by cecal ligation and perforation (CLP) surgery, with a single puncture of the cecum using a 14G needle. Naïve (n=52) or animals that survive 5 (n=24) or 10 days (n=27) after the surgery were submitted to injection of LPS (i.v.1.5 mg/kg n=24) or immobilization stress (n=27). After 60 min, blood was collected for plasma nitrate, cytokines and hormones (ACTH, corticosterone) and brain for hypothalamic cytokines and synaptophysin determination. All experimental procedure, were approved by Animal Ethical Committee of USP-RP

(protocol number 2017.1.124.58). Results: Five days survivor animals showed increased plasma nitric oxide (p< 0.001) and IL-1β levels (p<0.05) that were abolished in the 10 days survivors. No change was seen for plasma IL-6 either 5 or 10 days after sepsis. In the hypothalamus of both survivors the reverse was seen with IL-6 increased (p<0.01) while IL-1β did not show any alteration. Hypothalamic synaptophyfisin expression was reduced in both survivors (p<0.05). and did not change after any stimuli.As expected, only the LPS administration, increased plasma and/or hypothalamic inflammatory mediators (NO, IL-1β, IL-6) levels in both groups (naïve and survivors) being apparently lower in the survivors. Surprisingly, there was no difference in the secretion pattern of ACTH and corticosterone between naïve and sepsis survivors submitted to immune challenge or immobilization stress. Conclusion: The hypothalamus-adrenal is already recovered soon after 5 days of sepsis induction responding with a normal secretion of ACTH and corticosterone when required.
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SL612CB

Zinc deficiency and high fat diet during growth predispose to metabolic disorders in adult life

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Introduction: Zinc deficiency can coexist with overweight and obesity during growth predisposing to metabolic disorders in adult life. Objective: We evaluated if fetal and postnatal zinc deficiency exacerbates the extent of adiposity and metabolic dysfunction induced by high fat diet (HFD) in male adult rats. Methodology: Female Wistar rats received low (L:8ppm) or control (C:30ppm) zinc diets from pregnancy to offspring weaning. C male offspring continued with C(C) or HFD (60% of total calories)(CH) diets. L offspring were fed L(L) or L and HFD(LH) diets. At day 81, blood oxidative stress, glucose tolerance test (GTT), lipid profile, and morphology, adiponectin expression and oxidative state of retroperitoneal adipose tissue (RPAT) were measured. Two way ANOVA, Bonferroni post-test, mean±SEM,*p<0.01 vs C, †p<0.01 vs L, ‡p<0.01 vs CH. N=8 per group. FFyB-UBA-CICUAL approval Exp 0061021/18.Res 4370.Results: CH and LH showed higher bodyweight (C:418±13;CH:505±9*;L:401±10;LH:444±5‡†g) and RPAT weight. L, CH and LH had a decrease of adipose cells density and adipocytes hypertrophy (C:4958±388;CH:9621±586*;L:8130±448*;LH:11833±440‡†µm2). LH showed lower body weight and higher adipocyte area than CH.CH, L and LH rats showed decreased adiponectin ARNm expression (C:1,96±0,10;CH: 0,57±0,08*,L:0,68±0,07*;LH:0,73±0.07*(relative to Rplp0 gen), increased levels of lipid peroxidation (TBARS:C:0.21±0.02;CH:0.38±0.04*;L:0.38±0.04*;LH:0.43±0.04*pmol.MDA/mg.prot) and decreased levels of SOD and catalase antioxidant activities in RPAT.LH and CH showed an increase of GTT curve area (C:27797±504;CH:30827±971*;L:27826±809;LH:34851±1344‡† min.mg/d). L, LH and CH showed higher plasma glucose levels after 3 hours of glucose overload. Zinc deficiency exacerbated alterations induced by HDF. HFD and zinc deficiency increased triglycerides concentration, plasmatic TBARS levels (C:1,7±0.2;CH:2,8±0.2*;L:2,9±0.2*;LH:2,6±0.3*pmol.MDA/mg.prot) and catalase antioxidant activities in erythrocytes. Conclusion: Zinc deficiency and/or HFD during growth induce morphological and functional dysfunction of RPAT that could contribute to increase systemic oxidative stress, reduce glucose tolerance and alter lipid metabolism in adult life. Zinc deficiency during fetal and postnatal life exacerbates some of the alterations induced by HFD.
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KG687ML

Impact of chronodisruption during gestation on vascular function in the adult offspring.

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Introduction: A potential harmful condition for fetal development is gestational chronodisruption, a possible determinant for fetal programming related to modern 24/7 society, being an adverse condition for the fetus, the mother, and the adult offspring. Indeed, adult rats gestated under chronic photoperiod shifting (CPS) display increased heart rate variability and higher systolic blood pressure. However, the effects of chronodisruption during gestation on postnatal vascular physiology are still unknown.

Aims: To assess the vascular function in adult offspring gestated under CPS. Methodology: All animal care and experimental procedures were approved by the local IACUC. Pregnant rats were housed under 12:12 (LD, n=6) or chronic photoperiod shifting, from 0 until 18 days of gestation (CPS, n=6). After birth, all animals were raised in LD conditions. At 200 days old, we assessed femoral vasoreactivity by wire myography, performing concentration-response curves (CRCs) to KCl phenylephrine (Phe), and endothelin-1 (ET-1) as vasoconstrictors; and methacholine (MetCh), sodium nitroprusside (SNP), and melatonin (MEL) as vasodilators. Also, arteries were preserved for vascular morphometry analysis. All data were analyzed by a Mann-Whitney test, and a significant difference was considered when p≤0.05. Results: Femoral arteries from CPS males showed increased contractility (KCl phenylephrine, ET-1), compared with control adult arteries. Besides, vasodilator responses (MetCh, SNP, and Melatonin) were also higher in CPS animals. Moreover, CPS revealed vascular wall thickening associated with increased media layer (LD, 70.1±4.8 vs. CPS, 94.9±5.5 µm). Conclusions: Our results indicate that CPS programs vascular reactivity in male adult offspring. Although there is enhanced vasodilation, showing better endothelial function, an increased vasoconstrictor function associated with vascular remodelling may represent an initial hypertensive (compensated) phenotype.
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GH618NJ

Contribution of STIM-activated TRPC-ORAI Channels to the Intermittent Hypoxia-induced Pulmonary Hypertension

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Introduction: Obstructive sleep apnea, a breathing disorder characterized by chronic intermittent hypoxia (CIH), is associated with pulmonary hypertension (PH). Rats exposed to CIH developed pulmonary vascular remodeling (PVR) and PH, but the underlaying mechanisms are not well-known. Stim-activated Ca2+ TRPC-ORAI channels (STOC), are overexpressed in lung and play contributing to the development of PVR and PH in animals exposed to sustained hypoxia, and therefore, potentially involved in the pulmonary vascular alterations resulting from CIH. Aim: To assess the effects of 2-APB, a STOC blocker on the lung vascular remodeling and PH in rats exposed to CIH. Methods: Procedures were approved by the Scientific Ethical Committee for the animal and environment care from PUC de Chile. Sprague-Dawley rats (~200g) were exposed for 28 days to CIH (5% O2, 12 times/h for 8h). At 14 days of CIH, osmotic pumps containing 2-APB (10 mg/kg/day n=6) or its vehicle (n=6) were implanted, and the exposure to CIH continued for additional 14 days. At the end of CIH period, right ventricular systolic pressure (RVSP) was measured, and lungs were dissected to measure PVR and mRNA levels of the STOC forming subunits TRPC1, 4, 6, ORAI 1, 2 by qPCR. Animals were compared with aged mated controls (n=6). Values were expressed as average ± S.E. One-way ANOVA-followed by Newman Keuls. Results: The 2-APB treatment was associated to a lower RVSP (24.7 ± 1.6 mmHg) compared with non-treated animals (36.5 ± 1.1 mmHg), an effect also observed in PVR (66.3 ± 9.4 % vs 48.2 ± 3.5 %, 28d-CIH vs. CIH-2-APB, respectively). In addition, 2-APB treatment resulted in decreased levels of TRPC1, 4 and TRPC6, but increased ORAI 1. Conclusion: These results suggest that STOC plays a key role in the develop of the vascular remodeling and PH induced by CIH.
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GF973ND

Antimalarial drugs chloroquine and hydroxychloroquine cause negative inotropic and chronotropic effect in isolated heart and deregulation of intracellular Ca2+ homeostasis and currents by Cav1.2 channels in isolated ventricular cardiomyocytes.

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Introduction: Chloroquine (CQ) and Hydroxychloroquine (HCQ) are antimalarial drugs derived from “quinine”, used during the SARS-CoV-2 pandemic, as they inhibit inflammasomes [1]. This abstract reports their mechanisms of cardiotoxicity [2]. Objective: To characterize the effects of acute exposure to CQ and HCQ at the heart, trying to understand the molecular underpinnings related to their cardiotoxicity. Methodology. Guinea pigs were used following ethical standards with a protocol approved by “Comisión Honoraria de Experimentación Animal”(Exp.Nº071140-001464-12). Hearts were isolated by the Langerdorff coronary retro-perfusion technique. A transducer attached to the base of the papillary muscle recorded tension. Electrical activity was measured by Ag-AgCl electrodes. Cardiomyocytes were isolated by enzymatic methods [3]. Data were obtained by Axon products. Confocal microscopy was done with Rhodamine for Ca2+. Statistic tests between treated and not treated were not parametric (Mann-Whitney-Wilcoxon). The best fit of Hill´s equation to dose-response curves used nonlinear regression. Results are shown as mean +/- s.e.m (n=4). Results: Both drugs have a negative inotropic effect, being more marked with CQ than HCQ (IC50=5±2.1 µM y 50±17 µM respectively). Besides they showed negative chronotropic and an arrhythmogenic effect at higher doses, being these more pronounced in CQ (IC50=13±3.4 µM and 20±4.6 µM for CQ, IC50=26± 4.4 µM and 60±12.6 µM for HCQ). Ca2+ currents showed greater inhibition by CQ (IC50=12±3.2 µM, and IC50=56±13.2 µM for HCQ). Intracellular Ca2+ dysregulation was observed with lower CQ doses, with a decrease in intracellular Ca2+ which lasted longer times. Conclusions: Both drugs alter cardiac function

after acute exposure with CQ having effects more pronounced than HCQ. The negative inotropic effect could be explained in part by Cav1.2 blockage as intracellular Ca2+ homeostasis is affected by both agents. The negative chronotropic and arrhythmogenic effect may be due to Cav1.2 and If partial blockage.
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Antioxidant and vasodilator activities in isolated rat aorta of ethanolic extract of flowers of Cannabis sativa L.
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Chemically, Cannabis sativa L. contains many active compounds such as terpenes, flavonoids, alkaloids, and cannabinoids. Of the latter, about 120 types have been identified. The most important, according to their potency and activity, are ∆9-tetrahydrocannabinol and cannabidiol. Currently, this plant's therapeutic effects vary from conclusive evidence on chronic pain, multiple sclerosis, and treatment of nausea and vomiting induced by chemotherapies to moderate to mild evidence in epilepsy, anxiety, sleep disorders, and post-traumatic stress. There is evidence of in vitro antioxidant activity of seed extracts and sprouts of this botanical species. Besides, there is evidence that synthetic azacannabinoids have an antihypertensive effect on hypertensive rats. In this work, we evaluated the antioxidant and vasodilator potential activities of a standardized ethanolic extract of the flowering tops Cannabis sativa L. (ExCs). To assess antioxidant activity, we used the DPPH method obtaining an antioxidant capacity of 215.0% and 257.5% with ascorbic acid and hydroquinone, respectively, at an ExCs of 3 x 10-7 M. Vasodilator activity was evaluated in isolated rat aortic rings with and without endothelium, resulting in 10.1% relaxation with an ExCs of 3 x 10-8 M in both kinds of rings, evidencing that this effect is endotheliumindependent. This study supports the potential therapeutic use of the species.

Area: Translational Physiology

FP168TM

Mitochondrial calcium overload promotes energetic dysfunction and hypertrophy in the failing human heart

Sobrecarga de calcio mitocondrial promueve disfunción energética e hipertrofia en falla cardiaca humana

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Background: Impairment of intracellular Ca2+ homeostasis and bioenergetics is a prominent feature of the failing heart, but the underlying metabolic perturbations are poorly understood. Mitochondrial calcium transport is mediated by the Ca2+ uniporter (MCU) and till now the comprehension of its role in pathophysiology is limited by the complete lack of this molecular understanding. However, in light of the recent molecular identification of the MCU, new perspectives have been opened. Methodology: Here we measured the expression of MCU on failing human left ventricular tissue obtained at the time of orthotopic heart transplantation or left ventricular assist device (LVAD) insertion, against non-failing heart tissue samples and after LVAD removal (post-LVAD). Moreover, we characterized the role of MCU in cardiac myoblast cells with reduced expression of MCU under angiotensin II-induced hypertrophy. Mitochondrial function mitochondrial biogenesis and oxidative stress markers were assessed. T-Test, and 1-way Anova were performed, N=3-27. This study followed all regulations and approvals from the Institutional Ethics Committee. Results: Upregulated MCU (2.2 fold) was associated with poor prognosis in patients with HF. Moreover, the MCU expression positively correlated with a pathological remodeling in patients with HF, contrary to patients post-LVAD where MCU expression decreases 40%. Furthermore, in cardiac myoblast cells, silencing MCU improved cell viability and prevents calcium retention capacity impairment when cells were insulted chronically with Angiotensin II (ANGII). Additionally, silencing MCU blocks the angiotensin II-dependent transversal hypertrophy and cell death. Mechanistically we found that this MCU-dependent anti-hypertrophic effect was mediated by preventing ANGII-induced mitochondrial ROS overproduction and the changes in the phosphorylation status of transcription factor A and NFkB. Conclusions: Our findings suggest that MCU-induced mitochondrial Ca2+ uptake promotes ROS modulation of TFAM, thus contributing to angiotensin-dependent hypertrophy, revealing a novel mechanism underlying mitochondrial Ca2+-mediated pathological remodeling, and providing a potential pharmacological target for HF.

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BD444SR

Resveratrol preserves mitochondrial function by Sirt3 activation in a right ventricle dysfunction model

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Introduction: Pulmonary Arterial Hypertension (PAH) increases pulmonary vasculature pressure leading to right ventricular (RV) failure. Previously, we showed that resveratrol (RES) has anti-hypertrophic properties and improved RV function in PAH. However, underlying mechanisms are not fully understood. Of note, sirtuins modulation has been identified as critical mediators that induce cardioprotection. Objective: To elucidate the contribution of sirtuins in RV protection by RES in a monocrotaline-induced PAH rat model. Methodology. Internal Committee for Care and Handling of Laboratory Animals from Tecnologico de Monterrey approved all animal procedures (protocols 2017-006, 2019-019). PAH was characterized by echocardiography; myocyte function and mitochondrial membrane potential (ΔΨm) were evaluated by confocal microscopy; mitochondrial respiration was assessed using high-resolution respirometry and calcium retention capacity (CRC) by fluorescence; Western Blot and immunoprecipitation evaluated post-translational modification. Data are presented as percentages, n>3. Statistical significance was set at $p< 0.05$ after T-test, or 1-way ANOVA. Results: RES improved PAH RV function by echocardiography. PAH showed increased calcium-transient amplitudes, but cell shortening was decreased and less efficient with slower time to peak shortening (TTPS, 81%) and half relaxation (TTHR, 41%). PAH-RES group showed higher transient amplitudes and cell shortening, faster TTPS (22%), and maintained control THR. PAH decreased 47% ΔΨm, 26% mitochondrial oxidative phosphorylation, and 81% CRC, while PAH-RES preserved mitochondrial oxidative phosphorylation activity and improved CRC by 2.5 folds. Acetylated proteins increased by 59% in PAH; PAH-RES decreased acetylation by 13% and increased Sirt3 expression 3.5 fold. CyclophilinD (CypD) is a direct Sirt3 target when acetylated promotes mitochondrial transition and permeability pore opening. Hyperacetylation (3.6 fold) of CypD by PAH was

prevented by 51% in PAH-RES group. Conclusion: Preventing CypD hyperacetylation through Sirt3 partially contributes to preserving mitochondrial function, which holds an energetic cellular capability to maintain myocyte function. These findings provide new insight into the mechanisms underlying RES protection.
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GL227LP

Identification of the main perinatal complications associated with pregestational obesity attended on the Aconcagua Health Service between the years 2015 - 2017, Chile.

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Introduction: Obesity as an inflammatory state promotes different pathologies. An increase in obesity has been seen in occidental countries and in women of childbearing age. Further perinatal complications have been associated with women with pre-pregnancy obesity. Objective: To determine maternal obesity's association with the increased risk of perinatal complications in pregnant women of the Aconcagua Service from 2015 to 2017. Methodology: Cross-sectional, descriptive, and retrospective study. After approval of the Ethical Committee from Servicio de Salud Aconcagua, a total of anonymized data of 5978 pregnant women who had their delivery in the hospital San Camilo were analyzed. The data were pre-processed in Python; the statistical tests were performed in SPSS. The qualitative data analysis was performed in absolute frequencies and percentages, and the quantitative data using the mean and standard deviation (S.D.). The relationship of obesity as a risk factor for perinatal complications is analyzed using the Odd Ratio calculation. All significance is established with a value of $p<0.05$. Results: 28% of the mothers presented pregestational obesity. In this group of women, the 61.5% had cesarean delivery, and 29% presents three or more perinatal complications. The weight of the newborn is higher in obese pregnant women. Obesity is a risk factor for fetal macrosomia, gestational diabetes, hypertensive disorders of pregnancy, request for surgical sterilization, hypothyroidism, cesarean section, premature membrane rupture, and premature delivery. Likewise, pre-pregnancy obesity is a risk factor for three or more perinatal complications. Conclusions: Obesity is a risk factor for the main perinatal complications presented in pregnant women of the Aconcagua Health Service during 2015 - 2017.
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QP636TR

Organizational effects of prenatal exposure to testosterone excess and activational effects of chronic hyperandrogenemia on insulin sensitivity in a sheep model of polycystic ovary syndrome

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An ovine model of prenatal exposure to a testosterone excess (EPT) has been used to study the pathophysiology of polycystic ovary syndrome in women. This syndrome is characterized by hyperandrogenemia, which can reach the fetus reprogramming his/her development (first-hit, organizational effects), increasing the risk of metabolic diseases during adult life, including insulin resistance and T2D after new insults (second-hit, activational effects). The objective was to determine the ontogeny of insulin resistance in offspring at different stages of somatic development. For this, a group of pregnant ewes were injected with testosterone between the 30-120 gestational days (gd) and another group only received the vehicle in which testosterone was diluted. The study was carried out only in females at two-stages: the fetal stage (120gd; T-fetuses n=12; C-fetuses n=10) at the end of testosterone administration to ewes (first-hit); and at the post-pubertal stage (38 weeks-old; T-females n=6; C-females n=6) after chronic administration of 40mg of testosterone, twice a week, for 8 weeks (second-hit). At the fetal stage, metabolic-endocrine studies were carried out in blood obtained from jugular vein and in the post-pubertal stage, insulin sensitivity was evaluated through an intravenous glucose tolerance test (IVGTT). Statistical analyzes used were means comparison test and two-way ANOVA, the comparison of the results between groups is expressed as P value magnitude. All experiments were approved by the Bioethics Committee of the Faculty of Veterinary Sciences. T-fetuses presented hyperandrogenemia ($P<0.0001$), hyperestrogenemia ($P=0.0012$) and a decrease in plasma concentration of insulin ($P=0.0405$), IGF-II ($P=0.0071$) and adiponectin ($P=0.0172$) in comparison to C-fetuses. During postpuberty, after chronic hyperandrogenemia, T-females secreted more insulin ($P=0.0260$) during the IVGTT and ISI-Composite tended to be lower ($P=0.0568$) than C-females. In conclusion, EPT alters the

endocrine status of the female ovine fetus, which could lead to lower insulin sensitivity during adult life after a new hyperandrogenemia.
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FH546LD

Impact of swim exercise on ultrastructural changes in the rectus abdominis muscle of long-term mild STZ-induced diabetes pregnant rats

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Introduction: Gestational Diabetes Mellitus (GDM) is risk factor to the development of Pregnancy Specific Urinary Incontinence (PSUI) and skeletal muscle dysfunction, including rectus abdominis muscle (RAM). Experimental study showed ultrastructural changes in the RAM of long-term mild STZ-induced diabetes pregnant rats. Swim exercise is effective on prevention and treatment of GDM and may minimize the reported alterations. Aim: Analyze the impact of swim exercise on ultrastructural changes in the RAM of long-term mild STZ-induced diabetes pregnant rats. Methods: Ethics Committee on Animals Experiments nº007/2016. On the first day of life, Wistar female rats received Streptozotocin (100 mg/kg, subcutaneously). At 90 days of life each four females were mated overnight with one male rat for the maximum period of 15 days. The presence of spermatozoa in the vaginal smear was considered gestational day 0. The animals that presented glycemic level between 120-300 mg/dL and <120 mg/dL were distributed in Control – Sedentary (C), Control – Exercised (Cex), Diabetic – Sedentary (D) and Diabetic – Exercised (Dex) groups. The Cex and Dex groups started the swim exercise protocol on gestational day 0 until gestational day 20 (60 minutes/day, 6 days/week). On gestational day 21 the lower third of rectus abdominis muscle was obtained to ultrastructural analysis. The stained sections were examined using transmission electron microscope (3 samples/group). The analysis was qualitative, so no statistical tests were used. Results: All groups showed disorganized Z lines, intermyofibrillar mitochondria, organized triads and myelin figures associated with degenerated organeles. The main finding was the presence of sarcomeres disruption areas in D and Dex groups. Conclusion: Sarcomeres disruption areas observed in both diabetic groups reinforced the deleterious effect of diabetes and showed that the swim exercise protocol used in the present study did not reverse these alterations in the RAM of long-term mild STZ-induced diabetes pregnant rats
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LL178GB

Role of CD38 in cardiovascular performance and its use as a therapeutic target in Obesity-induced diastolic dysfunction

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Introduction: The transition from fatty acid oxidation to the utilization of other substrates that occur during development of heart failure leads to the decline in NAD/NADH ratio, which increases susceptibility to stress. CD38 is the main NAD+-consuming enzyme responsible for NAD+ levels regulation. Objective: To investigate the role of CD38 in cardiovascular performance and its potential use as a therapeutic target in obesity-induced heart failure. Methods: All protocols were approved by the ethic committee of the corresponding institution. Comparisons were performed by unpaired Student´s t test and 2-tailed p values < 0.05 were considered statistically significant. Results: When comparing adult Wild-Type (WT) and CD38 Knock-out (KO) male mice we observed that the latter had higher exercise performance, increased heart rate variability (HRV) and decreased heart rate (HR). When comparing WT and CD38 catalytically inactive (CI) mice we observed the same results. WT mice treated with an antibody which blocks CD38 activity showed improved exercise capacity and HRV. In CD38KO, CD38CI and antibody treated groups, NAD+ levels in hearts were increased. We also compared WT control and CD38KO mice treated with FK866 (Nampt inhibitor) and observed that NAD+ levels in heart decreased as well as exercise capacity. Then, we compared WT and CD38CI aged mice, fed with normal or high fat diet (ND or HFD). HFD decreased exercise capacity in WT mice but not in CD38CI mice. Similarly, HFD induced diastolic dysfunction seen as an increase in E/e' ratio only in WT mice while CD38CI were protected. Mechanistically, we observed that blockage of CD38 resulted in increased SERCA expression. Conclusion: CD38 plays a role in cardiovascular performance by regulation of NAD+ homeostasis. In a model of obesity-induced diastolic dysfunction NAD+-boosting by blocking CD38 activity led to protection by up-regulating SERCA expression.

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TK453DQ

Morphological changes on placentas from pregestational obesity and its relation to newborn weight

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Introduction: Obesity is a condition that when presented in a pregnant woman it may result in placental alterations. These alterations alter fetal nutrition affecting the weight of the newborn at birth. Objective: To evaluate the association between placental inflammation in pregestational obesity and the decreased weight at birth. Methodology: Transversal, correlational, and retrospective study. Placentas at term were obtained from the Clinical Hospital CHRISTUS-UC (Chile) from mothers with pregestational obesity (n=13) and normal weight (n=14). Samples were fixed in formalin buffer and stained with hematoxylin-eosin for cytomorphological analysis. Statistical tests (Student t -test, Chi-squared, and one-way ANOVA) were performed at SPSS. The qualitative variables were expressed in percentages and the quantitative in mean and standard deviation (S.D.). Significative results presented $p<0.05$. Results: Total increased weight was 22.6% and 13.7% at delivery in women with pre-pregnancy normal weight and obesity, respectively. In women with pregestational normal weight, 21% suffer excessive gestational weight gain, meanwhile in obese mothers were 69%. Newborn weights were similar between the groups. Chorangiosis was seen in 54% of placentas from women with pregestational obesity, versus 21% in women with normal weight. Pregnant women with pre-pregnancy obesity showed an increase in the thickening of blood vessels in placentas from male compared to female fetuses (80% and 13%, respectively) ($p=0.015$). Placentas from women with pre-pregnancy obesity and adequate gestational weight gain showed a higher number of syncytial knots per field (23 ± 3), compared to those with excessive weight gain (15±4) ($p=0.026$), and the normal weight with adequate gain (14±3) ($p=0.013$). Moreover, placentas from women with pre-pregnancy normal weight with newborn >p90 showed increased syncytial knots compared to the newborn <p90 (24±2 and 14±2, respectively) ($p=0.031$). Conclusion: Pre-pregnancy obesity leads to excessive gestational weight gain altering the placental histomorphology. As for the relationship with the newborn weight, the results are not conclusive.
FONDECYT 1190316, Chile.

RJ848QJ

Current technologies for remote monitoring of gestational diabetes mellitus: a systematic review

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Introduction: One out of eight pregnant women of the medium social-economical level have gestational diabetes mellitus (GDM). In general, 5 to 10% of the women suffering from GDM develop type 2 diabetes after birth. Various technological tools have emerged to make these tasks more straightforward and more comfortable to achieve glycemic goals and facilitate constant glucose monitoring. Objective: To understand the impact of current technologies that assist patients with GDM in achieving glycemic goals. Methods: Using PRISMA methodology, a database search was carried out about the disease "gestational diabetes mellitus" and the parameter "glucose" to find "technological tools" and their impact on patients with GDM published during the period 2016-2020.Results: 17 articles were obtained in the databases (WoS, PubMed, and Scopus) to be analyzed. The primary measurement was glycemia, and four medical devices were found (glucometer: conventional, with an infrared port, with bluetooth, smart type, and continuous glucose monitor), which together with the digital technology, allowed specific functions through 2 identified digital platforms (mobile applications and web pages). In three articles, the 2-hour postprandial glucose was lower in the GDM groups with telemedicine than in the control group. In three articles it is concluded that there were no statistically significant differences in glycemia measurements. In three articles it is reported a high user satisfaction, comfort with use, and high utility on telemedicine. One report suggested a reduction in direct costs and medical visits thanks to telemedicine and no differences regarding costs and medical help. Conclusion: There are no negative aspects or evidence of harm, but more studies are needed to confirm the benefits of telemedicine in pregnant women with GDM.

KG435GG

Seroconversion and abundance of IgG antibodies againts S1-RBD of SARS-CoV-2 in recovered patients in Santiago.

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Introduction: COVID-19 is a pandemic caused by SARS-CoV-2 and has affected more than 30 million people around the world. In Chile, almost half a million were infected by SARS-CoV-2 and more than 13,000 patients have died from COVID-19. Objective: As part of the clinical trial NCT04384588, this work aimed to quantify the abundance of IgG against the S1-RBD fragment of SARS-CoV-2 (anti-RBD) in convalescent people from the Santiago area and evaluate their suitability as COVID-19 convalescent plasma donors. Methodology: We established a quantitative anti-SARS-CoV-2 IgG ELISA using S1-RBD as antigen and determined the neutralizing antibodies (Nab) using a luminescent SARS-CoV-2 pseudo-type. Convalescent samples were analyzed by ELISA and compared with persons who had never been exposed to SARS-CoV-2. Groups were analyzed by Mann-Whitney test and One-way ANOVA and Tukey's multiple comparisons. All values were depicted as a geometric mean with 95% CI. This research was authorized by FALP Scientific Ethical Committee. Results: 72.9% of the convalescent population (468 of 639) showed seroconversion with a range between 5-55µg/ml of anti-RBD IgG and were suitable candidates for a donation of convalescent plasma based on a positive ELISA assay to 1:320 dilution. The cases were analyzed by sex, age and days after symptoms offset and did not show significant differences. Anti-SARS-CoV-2 Nab was slightly correlated with increased levels of anti-RBD IgG. Unexpectedly, individuals with a similar IgG concentration showed dramatic differences in the ability to neutralize the virus in vitro, up to 100 times more than others. Conclusions: We confirm that the majority of the Chilean group studied in this research developed anti-SARS-CoV-2 antibodies. The quantification of anti-RBD IgG in serum from convalescent plasma donors is necessary to increase the detection of neutralizing antibodies, and the antiviral activity of serum IgG may not correlate with a positive qualitative ELISA result at 1:320.

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RC992HJ

Low Handgrip Strength Is Associated with Higher Liver Enzyme Concentrations in US Adolescents

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Introduction: Nowadays much uncertainty exists regarding the relationship between liver enzymes concentrations, liver disease risk factors and muscular fitness among young. Objective: The purpose of this study was to determine the association between muscle strength and liver enzymes and liver disease risk among US adolescents. Methods: Data from the NHANES cross-sectional study (wave 2011 to 2014) was used. For the statistical analysis we used analysis of covariance (ANCOVA). Descriptive data are reported as means and standard deviation for continuous variables and numbers and percentages for categorical variables, and a total of 1,890 adolescents were included in the final analysis (14.5 ± 1.7 years old, 47.8% girls). Strength was assessed using a hand-held dynamometer, and the maximum strength was normalized to body composition parameters (normalized by body mass [NHSw], normalized by trunk fat [NHSt], and normalized by total body fat [NHSf]). Trunk fat and total body fat was assessed by dual energy X-ray absorptiometry. This study is exempt from formal Ethics committee approval, as it involves de-identified data freely available over the internet (http://www.cdc.gov/nchs/nhanes.htm). Results: Handgrip strength was inversely associated with higher values of aspartate aminotransferase (AST) and gamma glutamyl transpeptidase (GGT) in all estimations of muscle strength (NHSw, NHSf and NHSt) than those with intermediate NHSw and low NHSw ($p < 0.001$). Likewise, adolescents with intermediate NHSw by body weight presented lower AST and GGT than adolescents categorized with high handgrip strength ($p < 0.001$). Low handgrip strength groups for all estimations of normalized handgrip strength was associated with an increased odds of liver disease: NHSw (Odds Ratio [OR]=3.51; CI 95%=2.34-5.26); NHSf (OR=2.10; CI 95%=1.29-3.43) and NHSt [OR=2.10; CI 95%=1.32-3.35). Conclusions: In conclusion, this study suggests that US adolescents with low handgrip strength have higher values of liver enzymes as well as a higher prevalence of liver disease.

LB123MP

Variants of the ORL1 gene in subjects with global cardiovascular risk and acute myocardial infarction.

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Introduction: Cardiovascular risk is associated with generation of reactive oxygen species and oxidation of LDL. The biological effect of ox-LDL depends on the activation of the LOX-1 receptor, which contributes to the formation of atherosclerotic plaque. LOX-1 is encoded by the ORL1 gene and previous work identifies several single nucleotide polymorphisms (SNPs) that modulate its activation. Aim: Study the genotype and allelic frequency of OLR1 gene polymorphisms in a sample of patients with cardiovascular risk and acute myocardial infarction (AMI) of the Bío-Bío region. Method: A sample was selected from a population in the commune of Concepción (138 with cardiovascular risk and 22 AMI). The individuals were weighed and the body mass index was calculated. Glycemia, insulin, and lipid profile were determined. Genotyping was performed by DNA sequencing of the ORL1 gene. The Ethical Committee of Universidad de Concepción and Hospital Guillermo Grant Benavente of Concepción approved the protocol. Results: The individuals were classified as low (51,), moderate (51), and high (36) risk, and IAM (22). The patients with AMI, their average age of 60 years, and with troponin levels of 192,607 ± 44,641 ng/ml and CK-Total of 2,723±657 U/L. Sequencing analyzes demonstrated the existence of 11 SNP variants in the OLR-1 gene. Six of them associated with RCV and five that have not been previously described (rs4237962, rs2634156, rs17174598, rs3736233, rs3816844s). Significant differences were found in the allelic frequency of the SNP rs3736234. A high prevalence of the rs3736234 (71%) and rs3736235 (69%) variants were found in the low-risk population, while the rs2634156 (73%) and rs3736233 (41%) were more prevalent in the population with AMI. Conclusion: These results suggest that the use of genetic tools associated with the calculation of global cardiovascular risk and AMI could be more accurate for the identification of populations at risk for cardiac lessons.
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BH788PF

Peculiarities of the Eosinophilic Menincoencephalitis after the Introduction of the Giant African Snail in Cuba

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Introduction: Eosinophilic meningoencephalitis is an inflammatory infectious disease reported in Cuba since the eighties of the last century and now extended throughout American continent. It was produced by the parasite Angiostrongylus cantonensis. Objective: To determine if there are differences between the patients suffering eosinophilic meningoencephalitis before the introduction of the African giant snail and after the entrance of this invasive species. Method: 19 paired serum and cerebrospinal fluid samples belong to the seroraquiotec of Laboratorio Central de Líquido Cefalorraquídeo (LABCEL). Albumin and IgG were quantified by immunodiffusion technique. LCR Laboratorio (version 3.8.1) was employed to perform the Reibergrams.T-student test to compare media values was employed using MedCalc Software LTD (Ostend, Belgium) version 16.0. Results: The percentage of IgG synthesis among the patients with eosinophilic meningoencephalitis after the introduction of the African giant snail was larger than the other group but not statistically significative. 14 % of the patient was adults in comparison with the actual moment where 50% was patients with age superior to 18 years. IgG intrathecal synthesis percentage was larger than the actual patients, in spite not be a statistical significance, the average age was superior in patients linked to the African giant snail than the previously reported ones. Conclusions: Intrathecal synthesis response in patients associated with the African giant snail demonstrate more aggressively of the parasite and the larger mean age of the sick persons assure that those ones are the ones that manipulate and spread the mollusc.

MJ519FH

Impact of the use of new technologies on the management of pregnant women with type 1 diabetes mellitus

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Introduction: Two types of monitoring for the control of type 1 diabetes mellitus (T1DM) are available: self-monitoring of blood glucose (SMBG) and continuous glucose monitoring (CGM), which can also be used during pregnancy. However, the improvement in maternal and fetal health is not yet known. Objectives: To identify the impact of CGM on the control of HbA1c and glycemia in pregnant women with T1DM, compared to the use of SMBG. Method: Systematic review of the descriptive approach was carried out in the databases WoS, PubMed, and Scopus, including articles published from 2015 to 2020. The publications carried out

interventions in the use of CGM compared to SMBG in pregnant women with T1DM. Results were measured by blood glucose and HbA1c levels. The results are preliminary (pre statistical analysis).Results: Of a total of 311 articles found, 13 satisfied the criteria for inclusion and exclusion. Interventions based on CGM compared to SMBG showed HbA1c has the same performances on the reduced values, independent of the monitoring used. On the other hand, the glycemia showed a higher decrease with the use of CGM. Conclusions: A small sample size supports the effectiveness of CGM in pregnant women with T1DM. The measurement of glycemia can be considered over the measure of HbA1c for the follow-up of metabolic control. The empowerment of women about their pathology should be considered. It is expected that CGM will be considered as an option that may be preferred to the use of SMBG by maternal-fetal health specialists in pregnant women with T1DM.
FONDECYT (1190316), Chile.

LG111KB

New insights into the development of experimental model of Gestational Diabetes Mellitus

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Introduction: Gestational Diabetes Mellitus (GDM) causes gestational diabetic myopathy characterized by atrophy and deficiency in contraction of the Rectus Abdominis and Pelvic Floor muscles, leading to urinary incontinence (UI). To better investigate the pathophysiology of this disease using innovative tools, to develop non-invasive biomarkers and therapy, it is necessary to develop accurate experimental models that mimic the physiological conditions of GDM and gestational diabetic myopathy. Goals: To develop a more effective methodology for inducing GDM in rats, based on cross-parental care and induction up to 12h post-birth. Methodology: The study was approved by the ethics committee (1234/2017). After birth within 12 hours 48 females (Sprague Dawley® Specific Patogen Free) were Diabetes Mellitus (DM) induced by Streptozotocin 100 mg/kg in citrate buffer (70 mL/kg), injected subcutaneously, 10 female were control (only citrate buffer). After the induction, the entire litter (males and females) was returned to the mother until 10 day of life, then the males were separated. The glucose levels were measured by rapid test 5 days after induction and at 90 days of life, after the detection of DM the rats were mated and the glucose level in pregnancy was monitored using glucose tolerance test (GTT) on the 17th day of pregnancy, to confirm the GDM. Statistical method: Kruskal Wallis significance $p < 0.05$. Results: All females induced had glucose levels above 140 mg/dL (moderate diabetes), there were no deaths and the control group was normal. All pregnant rats showed altered glucose levels at GTT, demonstrating the GDM. Conclusions: We obtained a more effective method of inducing GDM with high effectiveness, due to parental care, where males and females were kept together (cross-parental care) different from conventional methodologies with male separation that leads to females deaths. In addition, induction within 12 hours after birth with the dose used was extremely effective.
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KR212HH

Effects of intermittent fasting with high-intensity interval training over body composition, lipid profile, and food consumption on obese subjects: A Pilot Study.

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In Chile, 74.2% of the population is overweight or obese (OECD, 2019), becoming a public health and economic concern. Meanwhile, high-intensity interval training (HIIT) and intermittent fasting (IF) have emerged as separate approaches to reduce body weight and improve metabolic health. We propose establishing a new IF protocol (5:2) combined with 6 weeks of HIIT to reduce body composition and cardiovascular risk factors in obese subjects (OB). Methods: Thirteen OB subjects (age 20-55 yrs, BMI ≥25≤ 39.9 kg/m2, mixed gender) were assigned either: 1) IF (IF, n=6): 15h fasting and 9h of free feeding in a 5:2 protocol or 2) IF combined with HIIT (IF-HIIT, n=7): 4 days a week on a static bicycle performing 10 repetitions of 1:1 minute intervals at ~90% HRmax. Before and after the protocol, body composition, biochemical profile, and dietary intake were measured. The UFT Ethics committee approved this study. The data are shown as mean ± standard deviation and analyzed by ANOVA, followed by Fisher's LSD posthoc test. Results: A ~2% decrease was observed in body weight and BMI, with a reduction in waist circumference (WC) in both groups ($p<0.01$). Notably, the IF-HIIT group did not lose lean mass ($p=0.1$), presented a greater reduction in waist/hip ratio (WHR, 1.8%), waist/height ratio (WHtR, 3.2%), LDL levels (19.8%), and an increase in TG levels (40%). As expected, this protocol generated a lower intake daily of calories (~20%) in both groups, without requiring a daily count. Conclusion: This new protocol produces a moderate caloric restriction close to 20% that induces a loss of body weight and BMI in OB subjects. Moreover, IF combine with HIIT reduces several cardiovascular risk factors, such as WC, WHR, and WHtR, and attenuate the loss of lean mass in OB subjects compared to IF alone.

TG752GT

The antiarrhythmic effect of inhibition of the mitochondrial calcium uniporter by Ru360 under catecholamine overload is partly due to mitochondrial function preservation.

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Introduction: Ventricular arrhythmias are a major cause of mortality in patients with a cardiovascular pathology. Catecholamines have been associated with the development of ventricular arrhythmias and no new intervention has demonstrated efficacy in reducing mortality since the use of β-blockers. Mitochondrial calcium transport has been deemed necessary to provide an adequate adrenergic response and constant adrenergic stimulation may lead to mitochondrial calcium overload with subsequent mitochondrial dysfunction. Inhibition of the mitochondrial calcium uniporter (MCU) have been described to reduce asynchronized contraction in cellular and animal models. Objective: Assess the effects that MCU inhibition by Ru360, a MCU inhibitor, has on ventricular arrhythmia incidence in a model of catecholamine toxicity and characterize mitochondrial function to describe the subcellular mechanisms involved. Methods: The study followed the national guidelines for laboratory animal use and care and was approved by the Animal Care and Use Committee. 12-15-week-old C57bl/6 male mice received via IV Ru360, and ECG baseline was recorded. Afterwards, Isoproterenol (400mg/kg) was administrated subcutaneously and ECG recording was kept for another 20 minutes. Finally, hearts were excised, and mitochondria isolated for further characterization. Data is presented as mean ± SEM. Fisher exact test was performed to evaluate arrhythmia incidence and one-way ANOVA followed by Holm-Sidak method for mitochondrial experiments and heart rate measurements. n=5-18. Results: Ru360 treatment prevented completely the development of ventricular arrhythmias. Mitochondrial Ca2+ transport inhibition preserved mitochondrial function and membrane integrity demonstrated by a higher respiratory control (12.251 ± 1.081 vs 7.318 ± 0.724) and calcium retention capacity (235.813 ± 10.246 vs 124.602 ± 20.895 nmol Ca2+/mg) which appears to be caused by a reduced oxidative stress as a trend to preserve reduced thiol groups was shown. Conclusion: Ru360 prevents arrhythmias by possibly reducing mitochondrial Ca2+ overload, preserving mitochondrial function and preventing ROS formation.

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HP745LQ

Effect of high lipoprotein(a) on platelet reactivity in individuals with and without coronary artery disease

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Introduction: High lipoprotein(a) [Lp(a)] in plasma is an important coronary artery disease (CAD) risk factor and different mechanisms underlying the atherothrombotic potential of Lp(a) have been proposed such as impaired fibrinolysis; however, if platelet reactivity may be involved remain uncertain. Objective: To investigate where increased Lp(a), defined as Lp(a) ≥50 mg/dL, could be associated with increased platelet reactivity. Methods: A total of 396 individuals in the presence (82.3%) and absence (17.7%) of obstructive CAD were divided into two groups according to Lp(a) concentrations with a cut-off value of 50 mg/dL. The association between Lp(a) and adenosine diphosphate (ADP)-induced platelet reactivity was evaluated using VerifyNow P2Y12 assay as the primary objective. Platelet reactivity was also induced by arachidonic acid and collagen-epinephrine (C-EPI) and assessed by Multiplate, platelet function analyzer 100 (PFA-100), and light transmission aggregometry (LTA) assays. Secondary analysis included the primary endpoint in individuals with and without CAD. The protocols for this research were approved by the Ethics Committee of the Clinical Hospitals, University of Sao Paulo Medical School. Results: Overall, 294 (74.2%) individuals had Lp(a) levels <50 mg/dL (median 13.2 mg/dL) and 102 (25.8%) had Lp(a) levels ≥50 mg/dL (median 83.5 mg/dL), P<0.001. Univariate analysis in the entire population revealed no differences in ADP-induced platelet reactivity between individuals with Lp(a) ≥50 mg/dL [249.4 ± 43.8 P2Y12 reaction units (PRU)] versus Lp(a) <50 mg/dL (243.1 ± 52.2 PRU), P=0.277. Similar findings were present in individuals with (P=0.228) and without (P=0.669) CAD, and regardless of the agonist used or method of analysis (all P>0.05). Multivariable analysis did not show a significant association between ADP-induced platelet reactivity and Lp(a) ≥50 mg/dL [adjusted OR=1.00 [(95% CI 0.99-1.01), P=0.590]. Conclusion: In individuals with or without CAD, Lp(a) ≥50 mg/dL was not associated with higher platelet reactivity. (These results were recently published in Salsoso et al., Adv Ther 2020)

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BJ275BJ

Effect of eccentric and concentric cycling changes on pulmonary and plasma oxidative stress and inflammation markers in stable COPD patients

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Exercise is a key part of pulmonary rehabilitation, nevertheless, exercise can induce oxidative stress and inflammation in patients with chronic obstructive pulmonary disease (COPD). The purpose of this study was to compare pulmonary and plasma markers of oxidative stress and inflammation after concentric and eccentric cycling bouts in individuals with COPD. Methods: Ten patients with moderate COPD level (68.3 ± 9.1 y; forced expiratory volume in 1 second = 68.6 ± 20.4% of predicted) performed 30 min of moderate-intensity concentric (CONC-M:50% maximum concentric cycling power output; POmax) and eccentric cycling (ECC-M:50% POmax), and high-intensity eccentric cycling (ECC-H:100% POmax) in a randomized order. Metabolic demand was monitored during cycling. Indirect markers of muscle damage were assessed before, immediately after, 24 and 48 h after cycling (muscle strength, muscle soreness, and creatine kinase activity). Plasma oxidative stress (malondialdehyde: MDA), antioxidant (glutathione peroxidase activity: GPx), and inflammatory markers (IL-6, TNF-α) were measured before and 5 min after cycling. Exhaled breath condensate (EBC) samples were collected before and 15 min after cycling and analyzed for hydrogen peroxide (H_2O_2), nitrites (NO_2^-), and pH. The UFT Ethics committee approved this study. The data are shown as mean ± standard deviation and analyzed by ANOVA, followed by Fisher's LSD posthoc test. Results: Metabolic demand was 40-50% lesser for ECC-M than CONC-M and ECC-H. Greater muscle damage was induced after ECC-H than ECC-M and CONC-M. MDA decreased immediately after CONC-M (-28%), ECC-M (-14%), and ECC-H (-17%), while GPx remained unchanged. IL-6 increased only after ECC-H (28%), while TNF-α remained unchanged after exercise. Pulmonary H_2O_2, NO_2^- and pH remained unchanged after exercise. Conclusion: Moderate Eccentric cycling seems to be an ideal training modality for COPD patients, due to lower metabolic demand and similar exercise-induced level of oxidative stress and inflammation markers compared to concentric cycling.

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Evaluate the effects of Gabapentin and Pregabalin in the treatment of Peripheral Neuropathic Pain in the patients with Diabetes

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Introduction: Peripheral Neuropathic Pain is a common neurological problem in patients with Diabetes. There are some anticonvulsants to control such problem including Gabapentin and Pregabalin. Aim: We evaluated and compared the effects of these medications in controlling the Neuropathic Pain in patients with Diabetes. Methods: In this study, we evaluated the effects of Gabapentin and Pregabalin in two separated groups of patients each contained 30 patients with type 2 Diabetes. Each group contained 30 female patients, aged between 40 to 60 years old and under Metformin therapy with an average A1C level of 6.5 upwards. In one group, Gabapentin was administered with an average dosage from 100 mg daily upwards until controlling the patients' symptoms and based on their tolerance. In another group, Pregabalin was administered with an average dosage from 75 mg daily upwards until controlling the patients' symptoms and based on their tolerance. In the duration of 3 months, we evaluated the patients' symptoms and the amount of pain reduction with Douleur Neuropathique 4 Questions (DN4), Neuropathic Pain Diagnostic Questionnaire. Results: In the duration of 3 months of treatment with Gabapentin and Pregabalin in each separated group, both patient groups showed decreased levels of pain and their questionnaire scores also were reduced suggesting that both treatments were effective. In the group that was treated with Gabapentin, there were 27 patients that their questionnaire scores were lowered and their pain symptoms were reduced but in the group that was treated with Pregabalin, there were 22 patients which their questionnaire scores were lowered and their pain symptoms were reduced. Conclusion: According to the results of this study, both Gabapentin and Pregabalin are effective to treat Neuropathic Pain in the patients with Diabetes although this study showed a relatively better effect of Gabapentin in alleviating and treating the Diabetic Neuropathic Pain.

Area: Hypothesis

QP347RK

Urinary excretion of renal tubular proteins in rats with obstructive cholestasis. Their potential as urinary biomarkers.

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Introduction: Obstructive cholestasis (OC) predisposes the kidney to renal dysfunction. In rats with OC of 21 h, renal function evaluated through traditional parameters, such as creatininaemia (Crpl), uraemia (Upl) and glomerular filtration rate (GFR), remains unchanged. However, after 72 h of biliary obstruction in rats, GFR is decreased. Our research group has worked in the search of urine biomarkers that could early indicate the presence of kidney dysfunction in other pathologies. Objective: The aim of this study was to evaluate the urinary excretion of renal tubular proteins Caveolin 1 (Cav-1), Caveolin 2 (Cav-2), Aquaporin 2 (AQP2) and Na-K-Cl cotransporter 2 (NKCC2) as potential urinary biomarkers to predict kidney alterations in OC. Methods: Bile duct ligation of 21 h (BDL, n=4) was performed in Wistar rats. Sham-operated rats served as controls (S, n=4). Protocols were approved by CICUAL-FCByF-UNR (Res. 657/2016). Upl, Crpl, and creatinine clearance (ClCr) as measure of GFR were determined. The protein excretions were evaluated by immunoblotting in urine of 21 h. The excretion of Neutrophil Gelatinase-Associated Lipocalin (NGAL), a novel urinary biomarker of kidney injury was also examined by immunoblotting. Results: Mean ± SE. Data were analyzed using an unpaired t test (*p<0.05). Upl(g/L): S=0.40±0.02 BDL=0.41±0.02. Crpl(mg/L): S=5.80±0.41 BDL=6.27±0.22. ClCr(mL/min.100g): S=0.43±0.01 BDL=0.47±0.02. %Cav-1: S=100±5 BDL=120±6*. %Cav-2: S=100±5 BDL=128±8*. %AQP2: S=100±10 BDL=88±7. %NKCC2: S=100±5 BDL=101±13. %NGAL: S=100±9 BDL=111±9. Conclusions: This is the first work to detect Cav-1 protein in urine of rats. The urinary levels of Cav-1 and Cav-2, significantly increased in OC of 21 h, could be postulated as early and non-invasive biomarkers with the potential to predict subsequent renal alterations in OC, since traditional parameters of renal function and urinary NGAL are not modified at this time point.

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LH957SM

Immune-dominant SARS-CoV-2 antigens as a tool for screening of specific antibodies in infected and recovered patients from COVID-19.

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Introduction: Coronavirus disease 2019 (COVID-19) is caused by the SARS-CoV-2, a beta coronavirus which have currently infected more than 30 million people around the world. Spike (S) and Nucleocapsid (N) proteins are two key structural proteins with a role in virus entry and replication respectively, and both proteins have been described as major antigenic determinants, promoting seroconversion and production of neutralizing antibodies. Objectives: As part of the clinical trial NCT04384588, the antibodies measurement is crucial. We proposed to evaluate the presence and specificity of serum antibodies against relevant antigens of SARS-CoV-2 based on structural and lineal exposed epitopes. The measurement of serum antibodies has provided crucial Methodology: Electrophoresis and immunoblotting were performed against recombinant Spike-domains (S1, S2 and S1-RBD) and full Nucleocapsid protein. IgG, IgA and IgM were evaluated in plasma and serum from never-exposed to SARS-CoV-2 people, active COVID-19 and convalescent patients. Finally in-house ELISA assays for each tested antigen were developed and validated. Statistical analysis were performed using GraphPad Prism software, v8.0. Groups were analyzed by Mann-Whitney test. All values were depicted as a Mean ± SEM. This research was authorized by FALP Scientific Ethical Committee. Results: The three isotypes IgM, IgA and IgG antibodies against S or N proteins were individually detected and they showed dramatic differences at the level of abundance, temporality and specificity by Western Blot and ELISA assays. ELISA sensitivity and specificity was variable depending on the antigen bound to the solid phase. Overall, Spike showed higher specificity than the Nucleocapsid, and comparable sensitivity for both antigens. Conclusions: Western blot and ELISA test confirmed the seroconversion after infection and allowed us to implement the analysis of antibodies in blood for research purpose. This work provides information for some laboratories with limited resources to apply exceptionally basic research tools for monitoring COVID-19 cases during pandemic.

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KH966QB

Insular dopamine and DOPAC increase in rats who live with depressed ones

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Introduction: Emotional contagion is an affective primitive tool through which an individual can synchronize their physiological, emotional and behavioral state with those of others. This mechanism has been described in both humans and animals, however, is a field mostly unexplored. Objectives: Determine the effects on euthymic rats caused by coexisting with depressed rats; such consequences were evaluated through behavioral tests and the quantification of neurobiological markers. Methodology: 40 adolescent female Wistar rats were selected and then divided in groups of 8 distributed in 5 boxes, with one box assigned as the control group. In each box, 6 animals were pharmacologically depressed using Reserpine (an amine depletor) and the remaining 2 rats were labeled as “socials”, only being injected with a vehicle. The experiment, conducted at the Biophysics lab (FVET- UdelaR) the lasted 60 days, at the end the animals were euthanized and insula and blood were extracted for further molecular testing. The protocol was approved by the ethics committee (CEUA-FVet-UdelaR). Variance analysis was used on the obtained data. Results: Dopamine and DOPAC concentrations were measured in insula by HPLC, revealing that depressed rats had significantly lower levels of dopamine (278,02±63,9 vs 685,8±112,9, $p<0,05$) and DOPAC (997,1±218,4 vs 1988,3±190,5, $p<0,05$) than controls, whereas the socials presented a tendency (446,6±74,02 vs 685,8±112,9, $p=0,08$) to have lower levels of dopamine than controls but also had significantly (1126,1±105,6 vs 1988,3±190,5, $p<0,05$) lower levels of DOPAC. On the other hand, T4 (tiroxin) was quantified in blood by RIA and its levels didn´t vary between the groups. Conclusion: Rats that live with depressed ones exhibit behavioural patterns as well as neurobiological markers similar to those, evidencing the existence and influence of emotional contagion. Currently, we continue to research other possible effects of emotional contagion towards the validation of these animal models.

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