European Council for Cardiovascular Research

European Council for Cardiovascular Research (ECCR) Virtual Meeting – Programme 24th-25th November 2023

Friday November 24th (All times are CEST/CET)

09:00-09:30 Welcome and Opening - Carmine Savoia, ECCR president (Italy)

09:30-11:00 **Session 1**

Chairs: *Matthias Barton (Switzerland), Muscha Steckelings (Denmark)*

(20 in each talk + 30 min general discussion)

- *Cardiorenal Protective Effects of Combining SGLT2 Inhibition, Endothelin Receptor Antagonism and RAS Blockade in Type 2 Diabetic Mice. Ander Vergara (Spain).*
- *Obesity and cardiovascular disease: mechanisms and therapeutic solutions. Massimo Volpe (Italy).*
- *Angiotensin-(1-5): A new hormone within the RAAS? Muscha Steckelings (Denmark).*

Discussion

11:00-12:30 **Abstract session 1: Presentations of selected abstracts**
9 ABSTRACTS (8 min presentation + 2 Discussion)

Chairs: *Ana M Briones (Spain), Marko Poglitsch (Austria)*

11:00-11:10 - ***A.F. Rodrigues:*** *Brain Local Angiotensin II Production Relies on Renin Activity.*

11:10-11.20 - ***Beatriz Delgado-Valero:*** *Let-7f-5p mediates galectin-3 renal deleterious effects through endoplasmic reticulum stress activation in obese rats with myocardial infarction.*

11:20-11:30 - ***Sthéfanie Chaves de Almeida Gonçalves:*** *Angiotensin-(1-7) protective effect on anxiety and depression-like behaviors in asthmatic mice.*

11:30-11:40 - ***Federico Bernardo Rossi:*** *The Water and electrolytes content in salt-dependent human HYpertension in the SKIn (WHYSKI) before and after surgical cure of primary aldosteronism.*

11:40-11:50 - ***Igor Maciel Souza-Silva;*** *Phosphoproteomics analysis reveals that angiotensin-(1-5) inhibits the mTOR signalling pathway in endothelial cells associated with anti-senescence effects.*

11:50-12:00 - ***May Fayad:*** *Role of the mineralocorticoid receptor in the physiology of the adrenal cortex and the development of aldosterone producing adenomas.*

12:00-12:10 - ***Oliver Thomas****: An analysis of sodium intake throughout adulthood on later-life cognition.*

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Friday November 24th continued: (All times are CEST/CET)

12:10-12:20 - ***Sahar Samanbar:*** *Angiotensin Type 2 Receptor (AT2R) stimulation reduces blood pressure and reverses endothelial dysfunction in a model of diet-induced obesity in mice.*

12:20-12:30- ***Samuel Adu:*** *Palmitoylation of NADPH Oxidase (Nox5) as a potential post-translational regulatory process*

12:30-13:30 **Interlude**

13:30-15:00 **Session 2**

Chairs: *Michael Bader (Germany), Gian Paolo Rossi (Italy),*

(20 min each talk + 30 min general discussion)

- *Aging impairs neurovascular interface in the heart. Stefanie Dimmeler (Germany).*
- *Sleep disturbance in cardiac disease: role of immune-mediated denervation of the pineal gland. Stefan Engelhardt (Germany).*
- *Mechanisms of Autophagy in Cardiomyopathy. Junichi Sadoshima (USA).*

Discussion

15:00-16:30 **Abstract session 2: Presentations of selected abstracts**
9 ABSTRACTS (8 min presentation + 2 Discussion)

Chairs: *Martina Cebova (Slovakia), Giacomo Rossitto (Italy)*

15:00-15:10 - ***Marta Martínez Casales:*** *Effect of Nrf2 deletion in cardiovascular alterations associated to Ang II-induced hypertension.*

15:10-15:20 - ***Domenico Bagordo:*** *Coronary flow reserve in primary aldosteronism.*

15:20-15:30 - ***Elvira Bragado García:*** *Mineralocorticoid receptor antagonism prevents. inflammatory, profibrotic and osteogenic factor increase in perirenal adipose tissue of diabetic rats with chronic kidney disease.*

15:30-15:40 - ***Livia L Camargo:*** *Vascular smooth muscle cell plasticity in human hypertension involves oxidative and ER stress.*

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Friday November 24th continued: (All times are CEST/CET)

15:40-15:50 - ***María Cuesta-Corral:*** *The impact of mitochondrial transplantation in cardiac damage associated with myocardial infarction.*

15:50-16:00 - ***Lídia Puertas-Umbert:*** *Rolipram impacts on redox homeostasis and cell signalling in an experimental model of abdominal aortic aneurysm.*

16:00-16:10 - ***Oreste Lanza:*** *Linking cardiac autonomic dysfunction, inflammation, and platelet reactivity in patients with acute coronary syndromes with or without coronary artery disease.*

16:10-16:20 - ***Stefano Bressa:*** *Retinal Wall to Lumen Ratio in patients with angina and no coronary artery disease.*

16:20-16:28 - ***Poster Presentation: Paloma Palma Guzmán;*** *Mineralocorticoid receptor antagonism reduces kidney damage and metalloproteinases MMP-2 and MMP-9 activities in diabetic rats with chronic kidney disease.*

16:30-18.00 **Session 3**

Chairs: *Marisol Fernandez-Alfonso (Spain), Koen Reesink (Netherlands)*

(20 min each talk + 30 min general discussion)

- *Magnesium signaling in health and disease. Rhian M Touyz (Canada), Francisco Rios (Canada).*
- *Microtubule network on arterial contractility. Thomas A Jepps (Denmark).*
- *Angiotensinogen Suppression: A New Tool to Treat Cardiovascular and Renal Disease. Jan AH Danser (Netherlands).*

18.00-18.15 **Art in Science Session**

18:15 **ECCR Annual business meeting – Members only**

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Saturday November 25th (All times are CEST/CET)

09:00-10.30 ***Mindshift Network Forum***
Including 6 ABSTRACTS (8 min presentation + 2 Discussion)

Chairs: *Thomas Unger (*Germany), *Maria Christina Zennaro (France)*

09:00-09:10 - ***Dellaneira Setjiadi:*** *Inappropriate Pulse Wave Velocity to Blood Pressure Level: Definition and Clinical Determinants.*

09:10-09:20 - ***Hala Ajjour:*** *Assessment of dynamic Angiotensin II (Ang II) -induced calcium signals in primary human adrenocortical cells.*

09:20-09:30 - ***Julius Soudant:*** *Interferon-stimulated gene 15 (ISG15) deletion protects against aldosterone-induced hypertension, cardiovascular and renal damage.*

09:30-09:40 - ***Ngoc Uyen Tran:*** *Mitigating the effects of accelerated vascular ageing in hypertension.*

09:40-09:50 - ***Maryam Jadoon:*** *Radiomics feature extraction for B-mode and radiofrequency images of the carotid arterial wall: a feasibility study*

09:50-10:00 - ***Nicolo' Faedda:*** *Vascular and hormonal interactions in primary aldosteronism.*

10:00-10:30 - Discussion and Forum activity

10:30-12:30 **Abstract Poster session 1: Presentations of selected abstracts**
11 ABSTRACTS (6 min presentation + 2 Discussion)

Chairs: *Ana M Briones (Spain), Christian Delles (UK)*

10:30-10:38 - ***Giuseppe Palmieri:*** *The impact of sex and dialytic age on sympathetic nervous system activation and cardiovascular risk in patients on chronic dialytic treatment.*

10:38-10:46 - ***Clement Byiringiro:*** *Effects of Macrolides on Vascular Cells and Blood Pressure Control.*

10:46-10:54 - ***Gianluca Baldini:*** *May Measure Month 2022 in Italy: Results of a Nationwide Survey.*

10:54-11:02- ***Domenico Bagordo****: Coronary sinus diameter to estimate congestion predict survival.*

11:02-11:10 - ***Carme Ballester-Servera:*** *Lysyl oxidase induced oxidative stress in calcific aortic valve disease and in atherosclerosis-associated calcification.*

11:10-11:18 - ***Matteo Lemoli:*** *Microcirculation and SARS-CoV2 infection: a follow up study.*

11:18-11:26 - ***Zoe González-Carnicero:*** *TLR4 is involved in IL-1β-induced effects. Role of Nrf2.*

11:26-12:04 - ***Alice Bongrani:*** *The lipolytic effect of zinc-alfa2-glycoprotein (ZAG) is related to its antioxidant activity: possible influence of glycosilation pattern.*

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Saturday November 25th continued: (All times are CEST/CET)

12:04-12:12 - ***Luigi Marzano:*** *SOPRANO Study Unveils Clinical and Biochemical Outcomes Post-Adrenalectomy for Primary Aldosteronism in Reference Centers.*

12:12-12:20 - ***Maria Fernanda Fussi:*** *Sex differences in the response to ischemic acute kidney injury and C21 nephroprotection.*

12:20-12:28 - ***Martina Cebova:*** *Effect of the Nitric Oxide Donor Nicorandil on Biochemical Parameters After Experimentally Induced Myocardial Infarction.*

12:30: 13.00 **Interlude**

13:00-14.15 **Abstract Poster session 2: Presentations of selected abstracts**
9 ABSTRACTS (6 min presentation + 2 Discussion)

Chairs: *Marta Gil-Ortega, (Spain) Francisco Rios (Canada)*

13:00-13:08 - ***Muhammad Saad Salman:*** *Dietary sodium over the adult lifecourse and cardiac remodelling.*

13:08-13:16 - ***Raquel Delgado Sarafian:*** *Putative role of Notch3 in Brown and White Adipose Tissue.*

13:16-13:24 - ***Raisa Brito Santos:*** *Voluntary exercise exacerbates the fibrosis in the kidney induced by folic acid in male C57BL/6J mice.*

13:24-13:32 - ***Rita Ribeiro-Oliveira:*** *Angiotensin-converting enzyme inhibitory activity elicited by brewing peptides: influence of the oral route and vascular microenvironment.*

13:32-13:40 - ***S. Ruvira:*** *Alterations in mesenteric resistance artery function in females exposed to fetal undernutrition.*

13:40-13:48 - ***Safiya Abdi Shugri Ahmed:*** *Investigations on the molecular interactions of angiotensin-(1-5) with the angiotensin AT2-receptor for receptor activation.*

13:48-13:56 - ***Sara Jiménez-González:*** *The interaction between Galectin-3 and endoplasmic reticulum stress mediates the cardiac alterations associated with myocardial infarction in obese rats.*

13:56-14:04 - ***V. López-Miranda:*** *Effect of TLR4 blockade on cardio-metabolic and renal alterations caused by diet induced metabolic syndrome. Modulation of the TLR4/MYD88/NLRP3 axis expression.*

14:04-14:12 - ***Alejandro Montoro-Garrido:*** *The use of mitochondrial transplantation on the management of renal complications associated with cardiorenal syndrome.*

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Saturday November 25th continued: (All times are CEST/CET)

14:15-15:45 **Session 4**

Chairs: *Christian Delles (UK), Carmine Savoia (Italy)*

(20 min each talk + 30 min general discussion)

- *Oxidative stress in hypertension and cardiovascular disease. Augusto Montezano (Canada).*
- *Inflammatory Signature of Human Hypertension: From Regulatory to Exhausted T Cells. Tomasz T Guzik (UK).*
- *Hypertension, Immune system, Neurovascular Dysfunction, and Cognitive Impairment. Lorenzo Carnevale (Italy).*

Discussion

15:45-16:15 **Key point lecture**

Introduction: Carmine Savoia (Italy)

- *Cardiovascular research and publishing in the era of AI. Michael Ryan (USA).*

16:15-16:30 **Closing remarks.**

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We are pleased to announce that *Clinical Science* is working in partnership with the ECCR to host a call for papers from the 2023 annual ECCR meeting. Clinical Science is a high-impact journal linking basic science to disease mechanisms, which underscores the translational scope of the journal focused on publishing science that advances our understanding of disease pathophysiology, with cardiovascular research at its core.

Submit your full original research and reviews to *Clinical Science* before 30 June 2024, and published papers will be promoted as a collection highlighting the research presented and discussed at this year's ECCR meeting. *Clinical Science* is published by Portland Press on behalf of the Biochemical Society and is led by Editor-in-Chief Michael J. Ryan. Publishing with *Clinical Science* is completely free under a standard license, and there are no page fees, publication charges or colour fees. You can choose to make your article available under an open access license, and more information on article publishing charges can be found on the journal's website. Authors at participating institutions can publish open access with no author-facing open access fees, through our transformative Read & Publish offerings. Find out more and see the full list of participating institutions

CLINICAL SCIENCE

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Full list of abstracts in order of programme.

ECRs selected oral communications.

1. ***Author: A.F. Rodrigue[1,2];*** Co-Authors**:** O. Domening[3,] M. Poglitsch[3], N. Alenina[1,2] & M. Bader[1,2]. Affiliation(s): [1]Max Delbrück Center, Berlin, Germany; [2]German Center for Cardiovascular Research (DZHK), Partner Site Berlin, Germany; Attoquant Diagnostics GmbH, Austria. ***Title:*** *Brain Local Angiotensin II Production Relies on Renin Activity.* ***Introduction:*** *The blood-brain-barrier limits the traffic of all components of the renin-angiotensin system. Brain-localized production of angiotensin II (AngII) has been challenged because the rate-limiting enzyme, renin, is expressed at very low levels in the brain. Our group has developed a transgenic mouse line that selectively overexpresses rat angiotensinogen (Agt) in brain astrocytes (Agt-Tg) yielding detectable levels of AngII.* ***Aim/Methods****: To confirm that AngII is locally produced in the brain, a line overexpressing Agt in the brain but lacking peripheral Agt was generated (Agt-KO-Tg) by breeding Agt-Tg with Agt-deficient mice (Agt-KO). To determine if renin is involved in the local production of AngII in the brain, Agt-Tg were crossbreed with renin-deficient mice (Ren-KO), resulting in a mouse line overexpressing Agt specifically in the brain but lacking renin (Ren-KO-Tg). Whole brains and blood were collected from males and females aging between 15-22 weeks. Angiotensin peptides (AngI, AngII, AngIII and Ang1-7) were quantified using LC-MS/MS by Attoquant Diagnostics GmbH. Results: AngII was detected in the brain, but not in the blood of Agt-KO-Tg mice suggesting local synthesis. Interestingly, AngII was not detectable in brain samples of Ren-KO-Tg in contrast to their littermates Agt-Tg indicating that renin is essential for brain Agt processing into AngII. Moreover, AngI and AngII were not detected in the blood of Ren-KO-Tg as expected.* ***Conclusions:*** *Collectively, the results from mice with brain-specific Agt overexpression support the notion that the brain locally and renin-dependently produces Ang II. The specific involvement of central or peripheral renin remains to be determined.*

2. ***Author: Beatriz Delgado-Valero[1];*** Co-Authors: E. Jover García[2], S. Jiménez-González[1], A. Romero-Miranda[1], B. Ramchandani[3], F. Islas[4], A. Fernández-de Celis[2], N. López-Andrés[2], V. Cachofeiro[1,5], E. Martínez-Martínez[1,5]. Affiliation(s): [1]Departamento de Fisiología, Facultad de Medicina, Universidad Complutense de Madrid–Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM), Madrid, Spain; [2]Cardiovascular Translational Research, Navarrabiomed, Hospital Universitario de Navarra (HUN), Universidad Pública de Navarra (UPNA), IdiSNA, Pamplona, Spain; [3]Servicio de Cirugía Cardiaca Infantil, Hospital la Paz, Madrid, Spain; [4]Servicio de Cardiología, Instituto Cardiovascular, Hospital clínico San Carlos, Madrid, Spain; [5]Ciber de Enfermedades Cardiovasculares (CIBERCV), Instituto de Salud Carlos III, Madrid, Spain. ***Title:*** *Let-7f-5p mediates galectin-3 renal deleterious effects through endoplasmic reticulum stress activation in obese rats with myocardial infarction.* ***Background.*** *Despite the detrimental role of Galectin-3 (Gal-3) in cardiorenal syndrome, its effects on myocardial infarction (MI) in the context of obesity have not been evaluated.* ***Aim(s).*** *To evaluate the role of Galectin-3 in the renal damage linked to MI within the context of obesity as well as the possible mechanisms involved. Methods. Male Wistar rats were fed a high-fat diet for 10 weeks. At the sixth week MI was induced by ligation of the coronary artery and different treatments were administered: A Gal-3 inhibitor (MCP); an endoplasmic reticulum stress (ERS) inhibitor (4-PBA). A group of rats with sham operation and normal diet were defined as control.* ***Results.*** *Obese animals with MI presented cardiac and renal damage associated with an increase in Gal-3 and a decrease let7f-5p renal levels.*

The treatment with MCP, was able to prevent all these alterations and improved renal fibrosis, ERS activation, oxidative stress, inflammation and the let-7f-5p levels. Luciferase assays demonstrated a direct interaction between let-7f-5p and ERS through ATF6B. Overexpression of let-7f-5p in HK2 cells improved the deleterious effects of ERS activation. In addition, MI-HFD animals treated with PBA prevented all the renal alterations observed in the animals except the decrease in let-7f-5p mRNA levels. ***Conclusions.*** *The data show that Gal-3 exerts deleterious effects at renal level in obese animals with MI through a decrease of let-7f-5p which is associated with ERS activation. Gal-3 emerges as a potential therapeutic approach in the management of the renal consequences of MI in the context of obesity.*

3. ***Author: Sthéfanie Chaves de Almeida Gonçalves;*** Co-Authors: Gregório JF, Ferraz KS, Magalhães GS, Zebral SA, Kangussu LM, Fontes MAP, Rodrigues-Machado MG, Millán RDS, Haibara AS, Santos RAS, Santos MJC. Affiliation(s): Department of Physiology and Biophysics, UFMG; Department of Morphology, UFMG; Department of Chemistry, UFMG; Program in Medical Science, FCMMG. ***Title:*** *Angiotensin-(1-7) protective effect on anxiety and depression-like behaviors in asthmatic mice. Allergic asthma is often associated with anxiety and depression, comorbidities that make it difficult to control the disease. Angiotensin-(1-7) reduces airway inflammation in the experimental asthma model, in addition to having antidepressant and anxiolytic effects when administered to the central nervous system. In this study, we evaluated whether Ang-(1-7) could attenuate emotional behaviors and inflammation in asthmatic mice. Male Balb/c mice, 8-10 weeks, were subjected to an experimental model of allergic asthma. The animals were divided into groups: control, asthmatic and one treated with oral Ang-(1-7) [60 µg/kg] or ICV (50 ng/h). Lung inflammation was assessed by histology, while anxiety-like behavior and depression-like behavior were assessed using the open field test and the tail suspension test, respectively. We also quantified c-Fos immunostaining in the paraventricular nucleus of the hypothalamus (PVN). CEUA nº 290/2018. Asthmatic animals initially showed decreased locomotor activity in the open field test, but this decline in mobility was significantly improved by both oral Ang-(1-7) and ICV treatment. Furthermore, both oral treatment and ICV contributed to the attenuation of inflammatory cell infiltration in the lung. Asthmatic animals exhibited a significant increase in c-Fos expression in the PVN, which was attenuated by oral administration of Ang-(1-7). These results indicate that Ang-(1-7), administered ICV or orally, has combined anti-inflammatory, anxiolytic and antidepressant effects, highlighting the potential for the development of new formulations based on Ang-(1- 7) for the treatment of inflammatory diseases.*

4. ***Author: Federico Bernardo Rossi[2];*** Co Authors: Francesca Torresan[1], Ilaria Caputo[2], Giovanni Bertoldi[2], Eva kohlscheen[2], Brasilina Caroccia[2], Giacomo Rossitto[2], Maurizio Iacobone[1], Gian Paolo Rossi[2]. Affiliation(s): [1]Endocrine Surgery Unit, Department of Surgery, Oncology, and Gastroenterology; [2]Internal and Emergency Unit and Specialized Hypertension Centre, Department of Medicine DIMED; [3]Plastic Surgery Unit, Department of Neurosciences University of Padua, Padua, Italy. ***Title:*** *The Water and electrolytes content in salt-dependent human HYpertension in the SKIn (WHYSKI) before and after surgical cure of primary aldosteronism.* ***Background:*** *Primary Aldosteronism (PA) is the prototype of salt-dependent hypertension. Recent evidence points to skin Na+ as a key component of body sodium content. Aim. To investigate Na+, K+, water content and the lympho-angiogenetic transcription factor Tonicity Enhancing Binding Protein (TonEBP) mRNA in the skin of patients with PA.* ***Methods:*** *We measured Na+, K+, and water content (by chemical-physical methods) and TonEBP mRNA copy number (by droplet digital PCR) in skin biopsies from 35 patients (age 56±10 yrs, 40% women) with unilateral PA treated before surgery with a mineralocorticoid receptor antagonist (MRA) at doses that corrected hypokalemia, and off MRA after adrenalectomy.* ***Results:*** *The skin specimen dry weight (DW) obtained at surgery was higher than at follow-up ($p<0.001$) and correlated positively with Na+, K+, and water content (all $p<0.01$). Surgical cure of PA did not affect skin DW-adjusted Na+ content, but markedly increased DW-adjusted K+ (from 1.14±0.1 µg/mg to 2.81±0.27 µg/mg) and water content (from 2.92±1.4 mg/mg to 3.85±0.23 mg/mg) ($p<0.001$ for both). Moreover, PA patients exhibited higher TonEBP mRNA copy number ($p<0.001$) than healthy subjects.* ***Conclusion:*** *In patients with unilateral PA, MRA treatment at doses that controlled hypokalemia and blood pressure skin Na+*

content was not higher than seen after adrenalectomy. The increase of skin K+ content after curative adrenalectomy indicates a more prominent cell tissue K+ depletion than estimated from serum K+ levels. The increased skin TonEBP expression suggests enhanced skin Na+ lymphatic drainage that could contribute to the lack of skin Na+ accumulation in PA.

5. ***Author: Igor Maciel Souza-Silva[1];*** Co-Authors: R. Belal[1], A. F. Rodrigues[3], P. Jensen[4], L. A. Jakobsen[4], A. Nawrocki[4], L. Rodrigues-Ribeiro[5], L. Vitved[6], R. A. Santos[5], T. Verano-Braga[5], M. Bader[3], C. Peiró[7], C. Sumners[8], M.R. Larsen[4], U. M. Steckelings[1]. Affiliation(s): [1]Institute for Molecular Medicine, Department of Cardiovascular and Renal Research, University of Southern Denmark, Odense, Denmark; [2]Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; [3]Max Delbrück Center for Molecular Medicine, Berlin, Germany; [4]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark; [5]National Institute of Science and Technology in Nanobiopharmaceutics, Department of Physiology and Biophysics, Federal University of Minas Gerais (UFMG), Belo Horizonte, Brazil; [6]Institute for Molecular Medicine, Department for Cancer and Inflammation Research, University of Southern Denmark, Odense, Denmark; [7]Department of Pharmacology, Faculty of Medicine, Autonomous University of Madrid, Madrid, Spain; [8]Department of Physiology and Aging, University of Florida, Gainesville, USA. ***Title:*** *Phosphoproteomics analysis reveals that angiotensin-(1-5) inhibits the mTOR signalling pathway in endothelial cells associated with anti-senescence effects.* ***Background:*** *Recently, we characterised angiotensin-(1-5) [Ang-(1-5)] as a novel, potent AT2R-agonist within the protective arm of the RAS. Signalling mechanisms induced by Ang-(1-5) are still largely unknown.* ***Objective:*** *To elucidate Ang-(1-5) signalling patterns in human aortic endothelial cells (HAEC)* ***Methods:*** *HAEC were treated with Ang-(1-5) (1µM) for 1, 3, 5 or 20 minutes and changes in protein-phosphorylations determined by quantitative phosphoproteomics and subsequent bioinformatics analysis. Key phosphoproteomics findings were validated by Western blotting. Effects of Ang-(1-5) on cell cycle regulation were studied by flow cytometry-based cell cycle analysis. A potential anti-senescence effect of Ang-(1-5) was investigated in HAEC rendered senescent by IL-1β through determination of mRNA expression of the senescence markers p21 and p53 by qPCR.* ***Results:*** *Analysis of phosphoproteomics data revealed Ang-(1-5)-induced dephosphorylation of the canonical mTOR targets Ser69-4EBP1 and Thr389-S6K1 in HAEC thus suggesting Ang-(1-5)-mediated mTOR inhibition. These dephosphorylations were confirmed by Western blotting. Ang-(1-5) treatment further counteracted Ser69-4EBP1 and Thr389-S6K1 phosphorylation induced by the mTOR activator MHY1485 thus providing additional evidence for an mTOR-inhibitory effect of Ang-(1-5). mTOR inhibition is linked to cell cycle arrest and protection against senescence. We indeed found that Ang-(1-5) led to cell cycle arrest and prevented the IL-1β-induced increase in the senescence markers p21 and p53.* ***Conclusions:*** *From our data we conclude that the endogenous AT2R-agonist Ang-(1-5) protects endothelial cells from senescence by inhibition of mTOR - a pathway that may further be exploited for the treatment of cardiovascular disease associated with endothelial cell senescence.*

6. ***Author: May Fayad[1,2];*** Co-Authors: Rami M. El Zein[1], Nicolo' Faedda[1], Bakhta Fedlaoui[1], Rita Chamoun[1,3], Parisa Rezvanisanijouybari[1,2], Sheerazed Boulkroun[1], Fabio L. Fernandes-Rosa[1], Eleanor Davies[2], Maria-Christina Zennaro[1,4]. Affiliation(s): [1]Université Paris Cité, Inserm, PARCC, Paris, France; [2]School of Cardiovascular and Metabolic Health, BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, United Kingdom; [3]Maastricht University Medical Center, Maastricht Limburg, The Netherlands; [4]Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Service de génétique, Paris, France. ***Title:*** *Role of the mineralocorticoid receptor in the physiology of the adrenal cortex and the development of aldosterone producing adenomas.* ***Introduction:*** *Primary aldosteronism (PA) is the major cause of secondary arterial hypertension. The mineralocorticoid receptor (MR) binds aldosterone and is expressed in the adrenal cortex specifically in the zona glomerulosa (ZG) and in aldosterone-producing adenoma (APA). Given the role of MR in tissue remodeling and its expression in the ZG and APA, we hypothesized that aldosterone could be involved in the pathophysiology of APA through MR by an autocrine paracrine mechanism. To investigate this hypothesis, we sought to identify the role of MR on adrenal cortex structure and*

function and regulation of aldosterone production in the adrenal cortex. ***Methods and results:*** *A mouse model was generated, expressing the Cre recombinase under Cyp11b2 promoter allowing specific expression of the Cre recombinase in the ZG. Those mice were crossed with Mrflx/flx mice. The adrenal phenotype of Cyp11b2+/Cre:Mrflx/flx (MRKOZG) mice was explored by morphological investigations. Genotyping of DNA from the adrenal cortex showed successful recombination in MRKOZG adrenals. MR expression was significantly reduced as shown by RT-qPCR and RNAscope. In MRKOZG mice, the adrenal cortex was disorganized and adrenocortical lineage appeared to be modified. In particular, ectopic expression of the fetal zone marker AKR1C1 in the zona fasciculata was observed in 12 weeks MRKOZG mice, suggesting abnormal transdifferentiation of adrenal cortex cells within the different zones.* ***Conclusion:*** *The identification of the underlying mechanisms of abnormalities observed in MRKOZG mice will allow to better understand cell lineage, differentiation and function of the adrenal cortex in relation to the development of PA.*

7. ***Author: Oliver Thomas;*** Co-Authors; Saadi Salman, Alun Hughes, Jane Maddock, Sarah James. Affiliation(s): University College London. ***Title:*** *An analysis of sodium intake throughout adulthood on later-life cognition.* ***Background:*** *Evidence from animal models and some observational studies suggests that sodium may have a direct association with cognitive impairment, however the evidence is contradictory. Aims: To examine the relationship between sodium intake over the adult life course and later-life cognition in a population-based birth cohort, and if these associations differ by sex.* ***Methods:*** *In 2283 participants (52% female) of the MRC National Survey of Health and Development born in a single week of 1946 in England, Wales and Scotland, multivariable regressions were used to analyse the relationship between dietary sodium intake (dNa) assessed from 5-day diet diaries at ages 36, 43, 53 and 60-4, and cognitive performance at age 69 (Addenbrooke's Cognitive Examination (ACE-III), visual search speed (VSS) and word learning memory test (WLT)). Models were sex-stratified, adjusted for confounders, and the potential mediating role of blood pressure was also analysed. Data are regression coefficients(β) with 95% confidence intervals, and false discovery rates were controlled at 5% using a Benjamini-Hochberg procedure.* ***Results:*** *After adjustment for confounders no convincing associations were observed between dNA at any age or average dNa across adulthood and ACE-III in either sex (average dNa, men: β=0.03(-0.58, 0.63)units/g, n =615; women: β=0.92(-0.14 (-0.85, 0.58)units/g, n = 688). Similarly, associations between dNa and VSS or WLT were weak and unconvincing at all ages. Adjustment for blood pressure did not significantly alter associations.* ***Discussion:*** *We did not observe any convincing relationships between dNa over the adult life course and later-life cognition in a UK population-based birth cohort.*

8. ***Author: Sahar Samanbar[1];*** Co-Authors: Daniel González-Moreno[1], Martín Alcalá[2], Marta Viana[2], Esther Carrera[1], María Larriva[2], José Miguel Cárdenas-Rebollo[3], Beatriz Somoza[1]*and Marta Gil-Ortega[1]*. Affiliation(s): [1]Departamento de Ciencias Farmacéuticas y de la Salud, Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, 28925, Madrid, Spain; [2]Departamento de Química y Bioquímica, Facultad de Farmacia, Universidad CEU-San Pablo, CEU Universities, 28925, Madrid, Spain; [3]Departamento de Matemáticas y Ciencias de Datos. Facultad de Ciencias Económicas y Empresariales. Universidad San Pablo-CEU, CEU Universities, 28925, Madrid, Spain. ***Title:*** *Angiotensin Type 2 Receptor (AT2R) stimulation reduces blood pressure and reverses endothelial dysfunction in a model of diet-induced obesity in mice.* ***Background:*** *Obesity constitutes one of the major risk factors for the development of cardiovascular alterations, including hypertension or endothelial dysfunction, among others. Previous studies of our group have revealed the potential of AT2R stimulation on preventing endothelial dysfunction and arterial stiffness induced by high-fat feeding.* ***Aims:*** *The purpose of this study was to elucidate whether AT2R stimulation could be able to reverse vascular alterations associated with obesity once they are established. Methods: Five-week-old male C57BL6J mice were fed a standard (Chow) or a high-fat diet (HF; 61% Kcal from fat) for 12 weeks. For the last 6 weeks, half of the mice of each group were also treated with a selective AT2R agonist, Compound 21 (C21, 1mg/kg in the drinking water) or vehicle, thus generating 4 groups (n=8/group); Chow-C, Chow-C21, HF-C and HF-C21. Blood pressure was assessed by the tail-cuff method at weeks 6, 9 and 12. The abdominal aorta was used for vascular reactivity experiments.*

Results: HF-feeding induced a significant increase in body weight ($p<0.001$) and blood pressure ($p<0.001$). In addition, relaxant responses to ACh (10-9-10-4M) and to Ang II (10-9-10-6M) were significantly impaired by the high-fat feeding ($p<0.05$). Although C21 did not modify body weight, it completely reversed alterations in blood pressure and relaxant responses to both ACh and Ang II, being the results observed in HF-C21 mice similar to the Chow-C group. ***Conclusion****: In light of these findings, we point out C21 as a potential therapeutic candidate for the treatment of vascular alterations derived from obesity.*

9. ***Author: Samuel Adu;*** Co-Authors: Rhian Touyz, Christian Delles, Will Fuller. Affiliation(s): School of Cardiovascular and Metabolic Health, University of Glasgow. ***Title:*** *Palitoylation of NADPH Oxidase 5 (NOX5) as a potential posttranslational regulatory process. NOX5 is a significant source of reactive oxygen species (ROS), which controls vascular tone and blood pressure, making it an interesting drug target for hypertension. Understanding the regulatory mechanisms of Nox5 is a high priority. We, therefore, investigate how palmitoylation as a posttranslational modification regulates Nox5 trafficking, localization and function. We engineered HEK cells stably expressing tetracycline-inducible wild-type and mutant (C338/342A) YFP-tagged Nox5. The substantial level of palmitoylation observed in the WT was abolished in the mutant Nox5. Confocal microscopy analysis indicated that Nox5 was predominantly localised in the ER, as previously reported. A small population of Nox5 also localises at the cell surface membrane. Membrane labelling and purification with biotinylation reagents revealed no significant difference in surface abundance between the WT and mutant Nox5. We evaluated Nox5 activity using the ROS sensor L-012 and detected no ROS in the absence of tetracycline. However, in tetracycline-treated cells, ROS were only detected following stimulation of cells with Nox5 agonists (400nM PMA and 1uM Ionomycin). The greater ROS generation observed in WT than in mutant cells indicates that palmitoylation enhances Nox5 activity. Next, using TurboID-mediated proximity labelling and mass spectroscopy, we identified Nox5 interaction partners, including palmitoylating (zDHHC5), depalmitoylating (APT2) enzymes and previously proven interactors. Evidence herein suggests that palmitoylation controls Nox5 activity and its surface membrane distribution. Further experiments will investigate the importance of identified palmitoylation enzymes, the effect of palmitoylation on Nox5 activity (and localization), and the downstream functional consequence of Nox5 palmitoylation in VSMC growth, migration, contraction, and inflammation.*

10. ***Author: Marta Martínez Casales;*** Co-Authors: Raquel Hernanz, Zoe González-Carnicero, María T. Barrús, Ángela Martín, Ana M. Briones, Ángel J. García-Yagüe, Antonio Cuadrado, María J. Alonso. Affiliation(s): [1]Dpto. Ciencias Básicas de la Salud, Fac. Ciencias de la Salud, URJC, Alcorcón, Spain. ***Title:*** *Effect of Nrf2 deletion in cardiovascular alterations associated to Ang II-induced hypertension.* ***Background:*** *Hypertension is a chronic inflammatory disease characterized by increased levels of proinflammatory cytokines and enzymes, leading to oxidative stress that contributes to the development of the cardiovascular alterations that characterize hypertension. The transcription factor Nrf2 (Nuclear Factor Erythroid 2-related factor-2) plays a key role in maintaining redox balance and has antioxidant and anti-inflammatory effects.* ***Objective:*** *To evaluate the participation of Nrf2 in hypertension as well as in the inflammatory state, oxidative stress and associated cardiovascular alterations.* ***Methods:*** *We used mesenteric resistance arteries, aorta and hearth from wild type and Nrf2-deficient mice infused or not with angiotensin II (AngII, 1.44 mg/Kg/day, 14 days). Results: Nrf2 deletion affected neither the left ventricle structure nor plasma and cardiac reactive oxygen species levels; however, it increased vascular oxidative stress, reducing nitric oxide (NO) bioavailability and leading to endothelial dysfunction. Despite not affecting AngII-induced hypertension, the absence of Nrf2 protected against development of cardiac oxidative stress, hypertrophy, and fibrosis. Additionally, it prevented hypertension-induced remodeling of resistance arteries and aortic endothelial dysfunction. These effects were associated with reduced oxidative stress and increased NO bioavailability and HO-1 expression. Furthermore, Nrf2 deletion did not affect the hypertension-induced increased of proinflammatory cytokine levels but avoided the induction of cyclooxygenase-2.* ***Conclusions:*** *In summary, Nrf2 deletion, although does not prevent hypertension development, may activate compensatory mechanisms, such as HO-1, which reduce oxidative stress; thus, it prevents*

cardiovascular alterations development that occur in this pathology, such as cardiac hypertrophy and fibrosis, vascular remodeling and endothelial dysfunction.

11. ***Author: Domenico Bagordo[1];*** Co-Authors: G. Rossitto[1,2]; A. Barchitta[3]; G. Nervo[1]; L. Ruzza[1]; G. Cabrelle[1]; C. Berton[1]; B. Caroccia[1]; F. Torresan[4]; M. Iacobone[4]; G.P. Rossi[1]. Affiliation(s): [1]Medicina d'Emergenza e Ipertensione, DIMED, Università degli Studi di Padova, Padova, Italy; [2]Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK; [3]UOC Clinica Medica 3, DIMED, Università degli Studi di Padova, Padova, Italy; [4]UOC Endocrinochirurgia, DISCOG, Università degli Studi di Padova, Padova, Italy. ***Title:*** *Coronary flow reserve in primary aldosteronism.* ***Background:*** *Primary aldosteronism (PA) features excess cardiovascular risk and microvascular dysfunction. It is not known whether this extends to the coronary vasculature and, as such, regresses after treatment.* ***Aims****: To investigate the presence of coronary microvascular dysfunction and its reversibility in PA. Methods Fifteen PA patients with lateralized aldosterone overproduction at adrenal vein sampling underwent Doppler echocardiography for the assessment of Coronary Flow Reserve (CFR: diastolic peak flow on the anterior descending artery during pharmacological [dipyridamole, n=14] or physical stress [n=1]/basal flow) and the ratio between diastolic peak flow and left ventricle mass (FMR), at the time of diagnosis (after withdrawal of RAAS-interfering drugs) and 4-6 months after specific treatment. In case of CFR ⩽2.5 and/or ischemia suspected during the stress protocol, a coronary CT angiography (coroCT) was performed.* ***Results*** *Out of 15 patients (5F; 51±9yo; BP:142/88 ±14/11 mmHg; sK+=3.7±0.4 mmol/L with KCL supplementation; DRC=3.0 [2.0-5.5] mIU/L; PAC=26.9 [15.0-51.5] ng/dl), 14 were cured by unilateral adrenalectomy (129/82±15/12 mmHg; sK+=4.3±0.4 mmol/L; DRC=11.1 [6.5-15.6] mIU/L; PAC=7.9 [5.2-9.0] ng/dl, p<0.05 for all); in one patient MRA was started due to incomplete biochemical cure. At diagnosis, n=7 (46%), n=3 (20%) and n=1 (7%) patients showed CFR ⩽ 3.0, 2.5 and 2.0, respectively; none had evidence of macrovascular disease at coroCT. Paralleling the reduction in left ventricle mass (trend; p=0.084), both CFR and FMR significantly increased after treatment (CFR=3.1 [2.6-3.3] vs. 3.4 [3.1-4.6], p=0.013; FMR=0.71±0.33 vs. 0.91±0.32 cm x g/sec x m2, p=0.038).* ***Conclusions*** *PA treatment improves coronary microvascular function, as assessed by established prognostic indices.*

12. ***Author: Elvira Bragado García;*** Co-Authors: Francisco Javier Manzano Lista[1], Elena Vega Martín [1], Marta Sanz Gómez[1], Reinhold Kreutz[2], Maria S. Fernández-Alfonso[1]. Affiliation(s): [1]Instituto Pluridisciplinar and Facultad de Farmacia UCM, Madrid, Spain; [2]Charité-Universitätsmedizin Berlin and Berlin Institute of Health, Department of Clinical Pharmacology and Toxicology, Germany. ***Title:*** *Mineralocorticoid receptor antagonism prevents inflammatory, profibrotic and osteogenic factor increase in perirenal adipose tissue of diabetic rats with chronic kidney disease.* ***Background and objective:*** *Increased perirenal adipose tissue (PRAT) thickness is a new independent risk factor for chronic kidney disease (CKD) in patients. We aim to characterize the expression of proinflammatory, profibrotic, and osteogenic factors in PRAT of diabetic Munich Wistar Frömter (MWF) rats and the effect of eplerenone (EP), a mineralocorticosteroid receptor antagonist, on these factors.* ***Methods:*** *Type I diabetes was induced in MWF rats by streptozotocin injection (15 mg/kg, i.p.) and additional exposure to a high fat/high sucrose (HF/HS) diet (D group). Oral treatment with EP (100 mg/kg/day in HF/HS diet) in diabetic animals (D-EP) was compared with untreated D rats and untreated non-diabetic MWF rats (C).* ***Results:*** *After 6 weeks if diabetes induction, D and D-EP showed significantly elevated blood glucose levels, reduced glucose tolerance, water intake and urine volume compared to C. Blood pressure and pulse wave velocity were similar between C and D groups and reduced by EP treatment. PRAT amount was higher in the D group with no effect of EP. Il-1ß, Il-6, Tnfα, Tgfß, Bgalp, Alp, and Col1A1 were significantly higher in PRAT of the D group and reduced to control levels by EP treatment. Reduced expression of anti-inflammatory Il-10 and Runx2 in PRAT of D group was prevented by EP.* ***Conclusions:*** *Diabetes induces an upregulation of inflammatory, profibrotic and osteogenic factors in PRAT of MWF rats which is prevented by EP. All these beneficial effects are independent of changes in glycemic parameters or in the amount of PRAT.*

13. ***Author: Livia L Camargo,[1,2];*** Augusto C Montezano[1,2], Yu Wang[2], Francisco J Rios[1,2], Rhian M Touyz[1,2]. Affiliation(s): [1] Research Institute of the McGill University Health Centre (RI-MUHC), Canada; [2] School of Cardiovascular and Metabolic Health, University of Glasgow, United Kingdom. ***Title:*** *Vascular smooth muscle cell plasticity in human hypertension involves oxidative and ER stress.* ***Background:*** *Hypertension triggers oxidative stress and endoplasmic reticulum (ER) stress, which contribute to vascular remodelling. Nox5 is a key contributor to oxidative stress in vascular smooth muscle cells (VSMCs), however, the relationship between Nox5-generated reactive oxygen species (ROS) and ER stress remains unclear.* ***Aim:*** *We investigated the contribution of Nox5 and ER stress in VSMC dedifferentiation during hypertension.* ***Methods:*** *VSMCs from resistance arteries of normotensive (NT) and hypertensive (HT) subjects were studied. Nox5 and IRE1 were silenced by siRNA. Nox5 compartmentalization (cell fractionation), ER stress activation (IRE1α, PERK phosphorylation) and differentiation markers (αSMA, SM22, MYOCD, KLF4, PCNA) were assessed by immunoblotting. ROS generation and inflammation/fibrosis markers were measured using chemiluminescence and ELISA.* ***Results:*** *Nox5 was upregulated in the ER fraction in HT subjects. IRE1α and PERK phosphorylation were increased in HT, an effect reduced by ROS scavenging (Tempol, 1mM) and Nox5 silencing. Conversely, ER stress (4-PBA, 1mM) and IRE1 (STF083010, 60μM) inhibition decreased ROS levels and Nox5 expression in HT, suggesting an interplay between Nox5 and ER stress in hypertension. VSMC markers αSMA, SM22, and MYOCD were reduced, whereas KLF4 levels were increased in HT. Expression of the proliferation marker PCNA was elevated, along with pro-collagen I production and release of the pro-inflammatory cytokines IL-6 and IL-8. Silencing of Nox5 and IRE1 reduced PCNA expression, pro-collagen I production, IL-6 and IL-8 production in HT.* ***Conclusions:*** *Our findings reveal a feed-forward relationship between Nox5 and ER stress, which contributes to VSMC dedifferentiation, an essential process underlying vascular dysfunction associated with hypertension.*

14. ***Author: María Cuesta-Corral[1];*** Co-Authors: A. Montoro-Garrido[1], A. Romero-Miranda[1], F. Islas[2], B. Ramchandani[3], V. Cachofeiro[1,4], E. Martínez-Martínez[1,4]. Affiliation(s): [1] Departamento de Fisiología, Facultad de Medicina, Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM), Universidad Complutense de Madrid (UCM), Madrid, Spain; [2] Servicio de Cardiología, Instituto Cardiovascular, Hospital Clínico San Carlos, Madrid, Spain; [3]Servicio de Cirugía Cardiaca Infantil, Hospital La Paz, Madrid, Spain; [4]Ciber de Enfermedades Cardiovasculares (CIBERCV), Instituto de Salud Carlos III, Majadahonda, Spain. ***Title:*** *The impact of mitochondrial transplantation in cardiac damage associated with myocardial infarction.* ***Background****: The heart demands a great deal of energy to maintain its contractile activity. This is obtained from mitochondria, which occupies 30% of cell volume. Myocardial infarction (MI) is associated with mitochondrial dysfunction and with cardiac structural and functional alterations. Previous studies have demonstrated the beneficial effects of mitochondrial transplantation in multiple models of acute damage, however, its application on chronic damage remains unknown.* ***Aims/Objectives:*** *To evaluate the impact of cardiac mitochondrial transplantation and the possible mechanisms involved in the pathological context of MI.* ***Methods:*** *Male Wistar rats underwent MI by ligation of the coronary artery. The sham group underwent the same procedure without occluding the artery. At that point, vehicle (PBS) or isolated mitochondria (180 µg protein, previously confirmed viability) were directly injected into the myocardium around the ligation to half of the animals of each group. After 4 weeks, cardiac function and structure were evaluated. Interstitial fibrosis, oxidative stress levels and markers of inflammation and endoplasmic reticulum (ER) stress were assessed in cardiac tissue.* ***Results****: The infarcted animals presented a reduction in systolic function together with cardiac hypertrophy an increase in both interstitial fibrosis and oxidative stress. Mitochondrial transplantation was able to prevent all these alterations after 4 weeks without modifying infarct size. In addition, it also improved the increase in markers of inflammation and ER stress activation.* ***Discussion/Conclusion:*** *Mitochondrial transplant emerges as a potential therapeutic strategy for cardiac damage associated with MI due to its beneficial effects on cardiac function, fibrosis, oxidative stress and ER stress activation.*

15. ***Author: Lídia Puertas-Umbert[1];*** Co-Authors: J. Alonso[1,2], M. Camacho[1], M. Sirvent[3], J. Martínez-González[1,2], C. Rodríguez[1]. Affiliation(s): [1] Institut d'Investigació Biomèdica Sant Pau, Barcelona, Spain; CIBER de Enfermedades Cardiovasculares, Instituto de Salud Carlos III, Madrid, Spain; [2]Instituto de Investigaciones Biomédicas de Barcelona-Consejo Superior de Investigaciones Científicas (IIBB-CSIC), Barcelona, Spain; [3]Hospital General de Granollers, Spain. ***Title:*** *Rolipram impacts on redox homeostasis and cell signalling in an experimental model of abdominal aortic aneurysm.* ***Background:*** *Cyclic nucleotide phosphodiesterases (PDEs) belonging to the PDE4 subfamily play a pivotal role in regulating the intracellular levels of cAMP, a second messenger crucial for vascular function. Our prior studies demonstrated the induction of PDE4B in human abdominal aortic aneurysms (AAA), and that pharmacological PDE4 inhibition limits aneurysm progression. Aims/objectives This study aims to underscore the mechanisms underlying the benefit of rolipram on AAA. Methods ApoE-/- mice were subjected to a 28-day angiotensin II (Ang II) infusion to induce AAA. PDE4 activity was inhibited using rolipram (3 mg/kg/day). Aortic diameter progression was assessed by ultrasonography. The expression of enzymes involved in redox homeostasis was analysed by real-time PCR and the activation of signalling pathways by Western blot.* ***Results:*** *PDE4B induction in human AAA was confirmed in a second cohort of patients, where this enzyme is primarily localised within inflammatory cells. In Ang II-infused ApoE-/- mice, rolipram elevated the percentage of aneurysm-free animals without affecting aortic rupture rates. PDE4 inhibition also mitigated Ang II-induced aortic collagen deposition. Furthermore, rolipram attenuated the upregulation of Nox2 triggered by Ang II, exacerbated Sod1 induction, and normalised Sod3 levels. Additionally, PDE4 inhibition reduced ERK1/2 activity and the induction of the canonical Wnt pathway in response to Ang II, while AKT activity remained unaltered.* ***Conclusion:*** *Our findings suggest that PDE4 inhibition by rolipram modulates redox homeostasis-related enzyme expression and influences critical cell signalling pathways associated with AAA development thus exerting beneficial effects on AAA. Funded by PI21/01048 and PID2021-122509OB-I00.*

16. ***Author: Oreste Lanza[1];*** Co-Authors: Antonio De Vita [2], Saverio Tremamunno [2], Armando Ferrera [1], Angelo Marino [2], Nello Cambise [2], Luca Proto[2], Dalila Tarquini [2], Anna Severino [2], Giovanna Liuzzo, [2], Gaetano Antonio Lanza[2], Emanuele Barbato[1], Carmine Savoia[1]. Affiliation(s): [1] Department of Clinical and Molecular Medicine, Faculty of Medicine and Psychology, Sant'Andrea Hospital, Sapienza University of Rome, Rome, Italy; [2]Fondazione Policlinico A Gemelli IRCCS, Department of Cardiovascular Medicine, Università Cattolica del Sacro Cuore Rome, Italy. ***Title:*** *Linking cardiac autonomic dysfunction, inflammation, and platelet reactivity in patients with acute coronary syndromes with or without coronary artery disease.* ***Background:*** *Inflammation is a contributor to atherosclerosis and acute coronary syndromes (ACS). Alterations in coronary microcirculation without coronary artery disease (CAD) may also trigger ACS, including myocardial infarction with non-obstructive coronary artery disease (MINOCA). An imbalance of cardiac autonomic nervous system (ANS) has been reported to contribute to vascular inflammation, endothelial dysfunction and platelet activation. Whether ANS dysfunction shows any association with ACS-related inflammation and platelet reactivity is still debated. Objectives: This study aims to investigate the relationship between ANS, inflammation, and platelet reactivity in patients with acute myocardial infarction with and without CAD. Methods: We compared 26 patients with non-ST-segment elevated myocardial infarction and obstructive CAD with 14 MINOCA patients. Patients underwent standard evaluations during hospitalization and follow-up. We also evaluated levels of inflammatory cytokines (IL-1β, IL-6, IL-18, TNFα), platelet activation by ADP, low-dose epinephrine and TRAP-6 stimulations. Cardiac ANS function was evaluated by Heart Rate Variability (HRV) using 24-hour ECG Holter recordings.* ***Results and Conclusions:*** *Cytokine levels and ANS function did not differ significantly between the two groups. A significant inverse correlation, was found between depressed HRV and IL-6 levels in both groups, highlighting the interaction between ANS function and inflammation in ACS. In MINOCA patients, platelet reactivity was increased compared to MI-CAD patients, even when considering patients on acetylsalicylic acid therapy only, but no relation with ANS activity was found. Our data confirm a relation of ANS activity with inflammation in ACS patients and suggest also an increased platelet reactivity MINOCA patients compared to those with MI-CAD.*

17. ***Author: Stefano Bressa;*** Co-Authors: S. Bressa, C. Agabiti Rosei, C. Bevacqua, P. Malerba, M. Lemoli, M. Baresi, F. Salvotti, C. Cattaneo, D. Rizzoni, C. De Ciuceis, M. L. Muiesan. Affiliation(s): Internal Medicine, Department of Clinical and Experimental Sciences, University of Brescia. ***Title:*** *Retinal Wall to Lumen Ratio in patients with angina and no coronary artery disease.* Introduction: *It is known that up to 50% of patients who undergo coronary angiography for typical chest pain have "normal" coronary arteries. Recently, therefore a third mechanisms of angina, consisting of the dysfunction of the coronary microcirculation has been added (micro-vascular angina). Ammonia PET is a new method for the study of coronary microcirculation.* ***Objective:*** *To evaluate the wall-to-lumen ratio (WLR) of the retinal arteries and the capillary density, indices of the structure of the microcirculation in patients with microvascular angina diagnosed by ammonia* ***Pet. Methods:*** *A total of 11 patients with negative angina pectoris who underwent coronary angiography or myocardial scintigraphy were enrolled in the study, 3 of whom had microvascular angina diagnosed by ammonia PET scan. The evaluation of the microcirculation was carried out by adaptive optics and capillaroscopy.* ***Results:*** *The WLR ratio was statistically significantly higher in patients with microvascular angina, as was the mean wall thickness (p 0.001 and p 0.015, respectively), compared to the control group. Correlation analysis showed an inverse correlation between the retinal arteriole WLR and the coronary flow reserve fraction in all patients.* ***Conclusions:*** *The greater WLR in patients with microvascular angina may be indicative of the presence of major alterations at the level of the microcirculation in both districts and this could support the hypothesis that the evaluation of alterations in the retinal microcirculation may represent an indirect and non-invasive mode of evaluation of the cardiac district.*

ECRs - Oral Mindshift communications.

1. ***Author: Dellaneira Setjiadi;*** Co-Authors: C. Delles; P. Boutouyrie; RM. Bruno. Affiliation(s): School of Cardiovascular and Metabolic Health, University of Glasgow, UK; Faculté de Médecine, Université de Paris, INSERM U970, Hôpital Européen Georges Pompidou, Assistance Publique Hôpitaux de Paris, France. ***Title:*** *Inappropriate Pulse Wave Velocity to Blood Pressure Level: Definition and Clinical Determinants.* ***Background:*** *Despite the established relationship between blood pressure (BP) level and hypertension-mediated organ damage (HMOD) severity, a disproportional degree of HMOD in relation to BP can be observed in some patients with hypertension.* ***Aims:*** *This study aims to quantify the proportion of population with inappropriate arterial stiffness measured by pulse wave velocity (PWV) and BP level including its associated characteristics.* ***Methods:*** *This observational monocentric cohort study utilised data from Hôpital Européen Georges-Pompidou (HEGP), Assistance Publique-Hôpitaux de Paris (APHP) that includes 5,186 (57.2% male, mean age 53 years) participants. 1,203 participants had a second PWV measurement. Residuals from log-transformed piecewise regression model for PWV prediction based on mean BP values were employed to define participants with inappropriate PWV. A multinomial regression analysis was conducted to find the associated clinical characteristics.* ***Results:*** *There are 522 participants with inappropriately high PWV and 517 participants with inappropriately low PWV, defined as 10th and 90th percentile of residuals. In the multinomial analysis, younger age, higher body mass index (BMI), and presence of diabetes mellitus (DM) are associated with inappropriately high PWV group. In participants with follow-up visit (n = 1,203), we found that classification into groups of inappropriate PWV was consistent, with 78% concordance.* ***Conclusions:*** *This study generates a classification method to define inappropriate HMOD compared to BP levels. Screening especially of younger patients for HMOD irrespective of their BP level may lead to early detection of organ damage and identifications of patients for targeted preventive strategies.*

2. ***Author: Hala Ajjour;*** Co-Authors: Livia Lenzini[1], Giorgia Pallafacchina[2], Brasilina Carrocia[1], Gian Paolo Rossi[1]. Affiliation(s): [1]Department of Medicine, DIMED, University of Padova; [2]Department of Biomedical Sciences, University of Padova. ***Title:*** *Assessment of dynamic Angiotensin II (Ang II) - induced calcium signals in primary human adrenocortical cells.* ***Background:*** *Angiotensin II (Ang II) and K+ stimulate aldosterone steroidogenesis by modulating intracellular calcium [Ca2+] i levels. We investigated [Ca2+]i dynamics of human aldosterone-producing adenoma cells (APA) and normal adrenocortical zona glomerulosa (ZG) (SN) by developing an analytical tool to decipher [Ca2+] i transients triggered by Ang II stimulation.* ***Methods:*** *APA and SN isolated CD56+ cells were exposed to increasing Ang II concentrations [10-10 to 10-7 M] at different extracellular [K+] [2.5 and 5 mM]. We recorded [Ca2+] i transients using Fura-2 dye employing live recording confocal microscopy. A peak detection pipeline was designed to analyze cell recordings with 3 peak parameters: amplitude, area under the curve, and full width at half maximum (FWHM).* ***Results:*** *We found that, with both K+ [2.5 mM] and [5 mM], basal [Ca2+] i levels were higher in CD56+ SN (N=16) than those of APA (N=13): at 2.5 mM [K+], SN = 0.87 a.u. vs APA=0.67 a.u. p= 0.023; at 5 mM [K+] SN = 0.85 a.u. vs 0.5 a.u. p= 0.028. The pipeline showed spontaneous oscillations in both cell types without significant differences in peak parameters. CD56+ SN and APA cells formed clusters after 24 hours in culture and they acquired a morphology reminiscent of native glomeruli after 72 hours. Alignment of recordings of cells residing in the same cluster revealed coordination in Ang II-evoked oscillations.* ***Conclusion:*** *The*

bias-free approach developed allowed to quantitatively compare [Ca2+] i transients in aldosterone-producing cells isolated from human adrenal cortex.

3. ***Author: Julius Soudant[1];*** Co-Authors: R. González Blázquez[1], M. Alcalá[2], M. Salaices[1,3,4], AB. García Redondo[1,3,4,5], AM. Briones[1,3,4]. Affiliation(s): [1]Departamento de Farmacología, Facultad de Medicina, Universidad Autónoma de Madrid, Spain; [2]Departamento de Química y Bioquímica, Facultad de Farmacia, Universidad CEU-San Pablo, Madrid, Spain; [3]Instituto de Investigación Hospital Universitario La Paz (IdiPAZ), Madrid, Spain; [4]CIBER Cardiovascular, Spain; [5]Departamento de Fisiología, Facultad de Medicina, Universidad Autónoma de Madrid, Spain. ***Title:*** *Interferon-stimulated gene 15 (ISG15) deletion protects against aldosterone-induced hypertension, cardiovascular and renal damage.* ***Background:*** *Interferon-stimulated gene 15 (ISG15) has been previously identified as a mediator of vascular damage in hypertension in response to Angiotensin II. Potentially, ISG15 modulates vascular remodelling, stiffness, and endothelial dysfunction via a post-translational modification (ISGylation), as well as in free protein form.* ***Objectives:*** *We aimed to understand how ISG15 impacts the cardiovascular and renal systems in aldosterone-induced hypertension.* ***Methods:*** *We used a 14-day aldosterone-infusion (600µg/kg/day) plus 1% NaCl drinking water model in wild-type and ISG15-/- mice. We measured blood pressure and changes in vascular function in the aorta and mesenteric resistance arteries (MRA) using tail-cuff plethysmography and wire myography, respectively. Additionally, histology, immunohistochemistry, and qRT-PCR were used to evaluate cardiac and renal structure, macrophage infiltration and gene expression of hypertrophy, fibrosis, and inflammation markers, respectively.* ***Results:*** *Aldosterone infusion increased the expression of ISG15 in the heart, aorta, and kidney of wild-type mice. The ISG15-/- mice were significantly protected from aldosterone-induced hypertension, cardiac and renal hypertrophy, inflammation, and fibrosis. In addition, ISG15 deletion protected the knock-out mice from aldosterone-induced endothelial dysfunction in the aorta and mesenteric resistance arteries.* ***Conclusions:*** *ISG15 is a novel mediator of cardiovascular and renal damage in aldosterone-dependent hypertension.*

4. ***Author: Ngoc Uyen Tran;*** Co-Authors: L. Schurgers, P. G. Shiels. Affiliation(s): University of Glasgow. ***Title:*** *Mitigating the effects of accelerated vascular ageing in hypertension.* ***Background:*** *A diseasome of ageing accompanies the growing global demographic of the aged, as increase in life expectancy has not been matched by health span (i.e. years of disease-free living). Within this diseasome, dysregulated ageing is a common underpinning component of different diseases, with hypertension accompanying vascular ageing. Senotherapeutic agents have been designed to mitigate the effects of cellular ageing and its associated pro-inflammatory secretome. Many such senotherapies remain restricted to a limited number of cell types and more broad spectrum agents are sought.* ***Objectives:*** *We aimed to evaluate the potential of two clinically approved anti-inflammatory/anti-hypertensive drugs (E30001 and TXA302) repurposed as putative senotherapeutic agents, to mitigate the effects of vascular ageing. Methods: Vascular Smooth Muscle Cell (VSMC) ageing was investigated in cells grown under both normative and morbid growth conditions using real time cell analysis (RTCA), transcriptomics, IHC, ICC and RT-PCR for a range of validated biomarkers of cellular ageing.* ***Results:*** *Both drugs tested exhibited senotherapeutic properties, potentially mediated by activation of Nrf2. E30001 displayed potential senolytic capabilities by activating apoptosis in senescent VSMCs via regulation of Serpin B1. Additionally, it enabled Nrf2-mediated downregulation of cell stress-related genes (NOXA, LMNA). TXA302 also appeared to be a putative Nrf2 activator, but its senotherapeutic effects were mediated by and induction of FOXO1- mediated antioxidant responses. Both compounds promoted cytoprotection by mitigating the effects of oxidative stress.* ***Conclusion:*** *Initial investigations on E30001 and TXA302 have successfully revealed senotherapeutic effects mediated through different biochemical pathways.*

5. ***Author: Maryam Jadoon;*** Co-Authors: F. Poli[1], H. Khettab[2], E. Bianchini[3], F. Faita[3], X. Jouven[1], P. Boutouyrie [1,2], J.P. Empana [1], R.M. Bruno [1,2]. Affiliation(s): [1]Université Paris Cité, Inserm, PARCC, F-75015 Paris, France; [2]Clinical Pharmacology Unit, AP-HP, Hôpital européen Georges Pompidou, F-

75015 Paris, France; [3]Institute of Clinical Physiology, Italian National Research Council (CNR), Pisa, Italy. ***Title:*** *Radiomics feature extraction for B-mode and radiofrequency images of the carotid arterial wall: a feasibility study. Background: Arterial ageing at the level of the carotid artery is characterized by arteriosclerosis and atherosclerosis. Carotid ultrasound can be used to obtain novel quantitative descriptors of arterial wall ultrastructure by radiomic techniques.* ***Objective:*** *The aims of this study are: i) to assess the reproducibility of radiomic features in describing carotid wall ultrastructure, with a focus on the influence of the selected Region-Of-Interest (ROI) size frame ii) to evaluate the predictive efficiency of different models using selected radiomic features for estimating chronological age.* ***Methods:*** *Radiofrequency signals from 200 individuals in the Paris Prospective Study 3 cohort were analyzed. Different data extraction settings were tested, including three end-diastolic frames and four ROI sizes. Three regression models (MRMR, Stepwise regression, Lasso L1) were compared using adjusted R-squared as the performance metric. Reproducibility across ROI sizes and frames was assessed with a t-test on residuals for age prediction. We evaluated R2 of three regression models (Random Forest, Support Vector Machine, Linear Regression) for predicting chronological age. Results: The MRMR model efficiently chose 13 features with consistent Mean Squared Error (MSE 31.0) and R-squared (R2 0.20). No significant variability was found across different ROI sizes and frames. Random Forest outperformed SVM and LR with a high R2 value of 0.96, compared to SVM (0.19) and LR (0.17).* ***Conclusion:*** *This feasibility study demonstrated that radiomic ultrasound features describe in a reproducible way carotid wall ultrastructure, with limited influence of ROI size and frame. Random Forest performs best in predicting chronological age using selected radiomic features.*

6. ***Author: Nicolo' Faedda;*** Co-Authors: Alaa Abdellatif, Bakhta Fedlaoui, May Fayad, Ilaria Del Gaudio, Eric Camerer, Ben Atkinson, Ana Maria Briones Alonso, Sheerazed Boulkroun, Maria-Christina Zennaro. Affiliation(s): Team 12/ PARCC/ Université Paris Cité/ INSERM. ***Title:*** *Vascular and hormonal interactions in primary aldosteronism.* ***Introduction:*** *Primary Aldosteronism (PA) is the most frequent form of secondary hypertension. As the adrenal cortex is a highly vascularized endocrine tissue, we make the hypothesis that adrenal vascular changes may modify adrenal cortex structure and function, creating a propitious environment for developing Aldosterone Producing Adenoma (APA).* ***Methods:*** *We crossed a new mouse model, expressing the Cre recombinase controlled by the Cyp11b2 promoter with either an mTmG mouse (Cyp11b2-Cre::mTmG) allowing to track adrenal cortex cell lineage, or with a Rarαfl/fl mouse model, which targets a pathway involved in adrenal cortex structure and vascularization. Mice were characterized under basal conditions, high or low salt diets (HSD/LSD) and dexamethasone (DEX) treatment, challenges that are known to modulate adrenal cortex plasticity and function.* ***Results:*** *Transdifferentiation and vascular development has been characterized in mice of both sexes until 12 weeks of age. After 2 weeks of DEX treatment, a significant reduction of ZF size, disorganization of cortex and vessels were observed with a strong decrease in corticosterone and Cyp11b1 expression. After three weeks of recovery, ZF and vessel regeneration was completed. Under HSD, Cyp11b2 expression was reduced, whereas under LSD, the ZG was expanded and Cyp11b2 expression increased. Additionally, salt diets had an impact on the transdifferentiation process. Characterization of the vasculature showed an increase in vessel area in the adrenal cortex while the vascular density was decreased, with a modification in vessel ramifications.* ***Conclusion:*** *Hormonal influence on the steroidogenic cell lineage, adrenal vascular network and of vascular changes on hormonal parameters, provides new insight in APA development.*

ECRs – Poster Presentations

1. ***Author: Giuseppe Palmieri1,2;*** Co-Authors: P. Nazzaro[3], A.R. Colavita[2], C. Savoia[1]. Affiliation(s): [1]Clinical and Molecular Medicine Department, Sant'Andrea Hospital, Sapienza University of Rome, Rome, Italy; [2]Cardiology Department, Antonio Cardarelli Hospital, Campobasso, Italy; [3]Nephrology Department, Antonio Cardarelli Hospital, Campobasso, Italy. ***Title:*** *The impact of sex and dialytic age on sympathetic nervous system activation and cardiovascular risk in patients on chronic dialytic treatment.* ***Background:*** *Kidney failure increases the cardiovascular risk through different mechanisms including sympathetic nervous system (SNS) activation. Nevertheless, the relation among dialytic age (DA), sex, SNS activation and cardiovascular risk remains elusive.* ***Aim:*** *We sought to study the possible association among DA, sex, and parameters of SNS activation.* ***Methods:*** *We enrolled 33 patients on chronic dialytic treatment (age: 65.5±14 years; dialytic age: 5.0±4.8 years, sex: male=20, female=13). Patients were sorted in two groups according to DA: group 1: DA <3.5 years (male=9, female=6); group2: DA >3.5 years (male=11, female=7). Before and after dialytic treatment, all patients underwent to the following evaluations: routine blood test, hemogasanalysis, ECG recording. The heart rate variability (HRV) parameter SDNN (Standard Deviation of NN intervals) was also assessed to evaluate SNS activation by Holter-ECG.* ***Results:*** *The groups resulted homogeneous regarding basal therapy, ultrafiltration rate (≈5% of pre-dialytic weight), biochemical analyses and ECG parameters before and after dialysis. SDNN was lower in patients with DA>3.5 years vs DA<3.5 years (89.0±24 ms vs 104±30 ms). Only males with DA>3.5 years had significantly lower values of SDNN vs male with DA<3,5 years (82±22 ms vs 110±34 ms; p<0.05), suggesting that only this group showed increased SNS activation which is known to correlate to higher cardiovascular risk.* ***Conclusion:*** *Only males with DA>3.5 years showed increased SNS activation and therefore increased cardiovascular risk. Thus, sex may differently impact on the cardiovascular risk related to DA through SNS activation in patients with chronic kidney disease on dialytic treatment.*

2. ***Author: Clement Byiringiro[1];*** Co-Authors: Ana Briones[2], Teresa M. Seccia[1], and Gian Paolo Rossi[1]. Affiliation(s): [1]Department of Medicine, DIMED, University of Padova; [2]Universidad Autónoma de Madrid. ***Title:*** *Effects of Macrolides on Vascular Cells and Blood Pressure Control.* ***Background:*** *Anecdotal reports showed a decrease in blood pressure (BP) values in subjects assuming macrolide antibiotics when co-administrated with calcium channel blockers. Recently, in the MAPA study (unpublished), a decrease in BP was observed in some patients with primary hypertension when given a single dose of macrolide. However, whether macrolides can induce per se vasorelaxation remains unknown. Aim: To investigate if macrolides induce vasorelaxation and identify the underlying mechanisms.* ***Methods:*** *The effects of 3 macrolides (roxithromycin, azithromycin, clarithromycin) were acutely investigated in isolated C57BL/6J mice aortas on wire myograph, following standard protocols. Phenylephrine pre-contracted aorta segments were exposed to different macrolide concentrations to measure relaxation/contraction. Longer-term effects of azithromycin (50 mg/Kg/d intraperitoneally for 14 days) were investigated in a mouse model of Ang II-induced hypertension. Blood pressure was measured with the tail-cuff method; functional and structural/mechanical properties of the aorta and mesenteric arteries were evaluated with wire or pressure myograph, as appropriate.* ***Results:*** *After exposure to macrolides (10-10 M to 10-5 M), phenylephrine pre-contracted mice aorta segments showed relaxation (maximum effect: -15-20% vs DMSO), with similar concentration-response curves. The vasodilating effect was abolished by the removal of endothelium*

and was blunted by L-NAME. The treatment with azithromycin lowered blood pressure values and also protected from Ang II-induced endothelial dysfunction. ***Conclusions:*** *Macrolides can induce relaxation in phenylephrine-precontracted mice aortas, via a NO-mediated pathway. In a model of Ang II-mediated hypertension, azithromycin not only lowered BP values but also exerted a protective effect on endothelial damage.*

3. ***Author: Gianluca Baldini;*** Co-Authors: Rita Del Pinto[1], Claudia Agabiti Rosei [2], Gianluca Baldini[1] , Claudio Borghi[3], Franco Cipollini[4], Santina Cottone[5], Giuseppe Antonio De Giorgi[6], Antonino Di Guardo[7], Maurizio Dugnani [8], Bruno Fabris[9], Cristina Giannattasio [10], Gilberta Giacchetti [11], Pietro Minuz[12], Giuseppe Mulè[5], Pietro Nazzaro[13], Gianfranco Parati[14], Marcello Rattazzi[15], Francesca Saladini[16], Riccardo Sarzani[17], Franco Veglio[18], Vito Vulpis[19], Claudio Ferri[1], Maria Lorenza Muiesan[2]. Affiliation(s): [1]University of L'Aquila, ESH Excellence Center, S. Salvatore Hospital, L'Aquila, Italy; [2]University of Brescia, ESH Excellence Center, Spedali Riuniti di Brescia, Italy; [3] Alma Mater Studiorum University of Bologna, ESH Excellence Center, Sant'Orsola-Malpighi University Hospital, Bologna, Italy; [4]Outpatients Hypertension clinic, P.I.O.T. San Marcello Piostoiese, San Jacopo Hospital, Pistoia, Italy; [5]University of Palermo, ESH Excellence Center, P. Giaccone Hospital, Palermo, Italy; [6]Outpatients Hypertension clinic, Lecce, Italy; [7]Outpatients Hypertension clinic, Catania, Italy; [8]Outpatients Hypertension clinic, Novara, Italy; [9]University of Trieste, Cattinara Hospital, Trieste, Italy; [10]University of Milano-Bicocca, ESH Excellence Center, Niguarda Hospital, Milan, Italy; [11]Azienda Ospedaliero Universitaria delle Marche, Ancona, Italy; [12]University of Verona, ESH Excellence Center, Policlinico G.B. Rossi, Verona, Italy; [13]University of Bari, Hypertension clinic A.M.Pirrelli, Bari, Italy; [14]University of Milano-Bicocca, St. Luke Hospital, Istituto Auxologico Italiano, IRCCS, Milan, Italy; [15]University of Padua, Hypertension clinic, Cà Foncello Hospital, Treviso, Italy; [16]Hypertension clinic, Cittadella Hospital, Padua, Italy; [17]Università Politecnica delle Marche, ESH Excellence Center, IRCCS-INRCA, Ancona, Italy; [18]University of Turin, ESH Excellence Center, AOU Città della Salute e della Scienza, Turin, Italy; [19]Emergency Medicine, Hypertension and Cardiovascular Risk Unit – Policlinico Hospital, Bari, Italy. ***Title:*** *May Measure Month 2022 in Italy: Results of a Nationwide Survey.* ***Introduction:*** *By affecting approximately 45% of the world's population, hypertension is the main risk factor for cardiovascular diseases (CVD). Of note, only half of hypertensive patients manage to have good blood pressure (BP) control. Relevant reasons for the persistence of uncontrolled BP during treatment are lack of compliance on the patients' side, and therapeutic inertia on physicians' side.* ***Methods:*** *During the global BP screening campaign "May Measure Month" (MMM) (May 1st to July 31st, 2022), a nationwide, cross-sectional, opportunistic study endorsed by the Italian Society of Hypertension was conducted on volunteer adults ⩾ 18 years to raise awareness of the health issues surrounding high BP. A questionnaire on demographic/clinical features and questions on the use of fixed-dose single-pills for the treatment of hypertension was administered. BP was measured with standard procedures.* ***Results:*** *A total of 1612 participants (mean age 60.0±15.41 years; 44.7% women) were enrolled. Their mean BP was 128.5±18.1/77.1±10.4 mmHg. About one in two participants was sedentary, or overweight/obese, or hypertensive. 55.5% individuals with complete BP assessment had uncontrolled hypertension; not being on a fixed-dose combination of antihypertensive drugs and not measuring their BP at home were common features in this setting. Self-reported adherence to BP medications was similar between individuals with controlled and uncontrolled BP (95% vs 95.5%).* ***Conclusions:*** *This survey identified a certain degree of therapeutic inertia and poor patients' involvement in the therapeutic process and its monitoring in the examined population, underlining the importance of prevention campaigns to increase risk factors awareness to reduce CV risk.*

4. ***Author: Domenico Bagordo[1];*** Co-Authors: A. Barchitta[1], G. Rossitto[1,2], L. Ruzza[1], D. Maio[3], G. Scaparrotta[4], F. Antonini Canterin[5], P. Piovesana[6], T.M. Seccia[1], F. Nalesso[4], L. Calò[4], G.P. Rossi[1]. Affiliation(s): [1]University of Padova, Emergency Medicine and Hypertension, University Hospital, Padova, Italy; [2]School of Cardiovascular & Metabolic Health, University of Glasgow, Glasgow, UK; [3]University of Ferrara, Cardiology, St Anna Hospital, Ferrara, Italy; [4]University of Padova, Nephrology, University Hospital, Padova, Italy; [5] Cardiology, Ospedale Riabilitativo di Alta Specializzazione di Motta

di Livenza (TV), Italy; [6]Cardiology, Ca'Foncello Hospital, Treviso, Italy. ***Title:*** *Coronary sinus diameter to estimate congestion predict survival. Background: Congestion predicts a poor prognosis but its assessment is challenging in clinical practice and requires a multiparametric approach. Aims: We investigated if the coronary sinus maximum diameter (CSmax) can estimate fluid overload/decongestion and predict mortality in a human model of rapid fluid unloading.* ***Methods:*** *In patients with end-stage kidney disease (ESKD) we measured by echocardiography the CS, and the inferior vena cava (IVC) for comparison, immediately before and after hemodialysis. Patients were prospectively followed up for all-cause mortality. Results: In the 60 recruited patients (age 76 [57-81] years, 40% female, left ventricular ejection fraction 57 [53-56]%) we found that HD-induced decongestion decreased the maximum diameters of both CS and IVC ($p \leqslant 0.001$ for all). CSmax was as accurate as the IVC maximum diameter and collapsibility for the identification of congestion, defined as pre-hemodialysis status (AUROC CSmax=0.902 vs IVC=0.895, p=n.s.). CSmax after hemodialysis > 9 mm predicted all-cause mortality at 12 months (Log-rank Chi square=11.49, p < 0.001).* ***Conclusions:*** *A persistently dilated CS after hemodialysis, is a marker of residual congestion and predicts death at one year in high-risk ESKD patients.*

5. ***Author: Carme Ballester-Servera[1,2];*** Co-Authors: J. Alonso[1,2], L. Cañes[1,2], P. Vázquez-Sufuentes[1], L. Puertas-Umbert[2], A. Fernández-Celis[3], M. Tauron[2,4], N. López-Andrés[3], C. Rodríguez[2], J. Martínez-González[1,2]. Affiliation(s):[1] Instituto de Investigaciones Biomédicas de Barcelona-Consejo Superior de Investigaciones Científicas (IIBB-CSIC), Barcelona, Spain;[2] CIBER de Enfermedades Cardiovasculares, Instituto de Salud Carlos III, Madrid, Spain; Institut d'Investigació Biomèdica Sant Pau, Barcelona, Spain;[3] Cardiovascular Translational Research, Navarrabiomed, IdiSNA, UPNA, Hospital Universitario de Navarra, Pamplona, Spain;[4] Departamento de Cirugía Cardíaca, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain. ***Title:*** *Lysyl oxidase induced oxidative stress in calcific aortic valve disease and in atherosclerosis-associated calcification. Background: Extracellular matrix (ECM) remodeling and oxidative stress are key players in cardiovascular calcification, an important prognostic factor of cardiovascular morbidity and mortality. Lysyl oxidase (LOX) activity determines ECM biomechanics, contributes to vascular oxidative stress and has been involved in vascular smooth muscle cell (VSMC) calcification.* ***Aim:*** *To assess the impact of LOX-derived reactive oxygen species (ROS) on valvular calcification and atherosclerosis-associated calcification.* ***Methods:*** *Studies were performed in human calcified aortic valves and valvular interstitial cells (VICs) exposed to osteogenic medium (OM) in the presence or in the absence of β-aminopropionitrile (BAPN), an inhibitor of LOX activity. Atherosclerosis was induced in transgenic mice that overexpress LOX in VSMC (TgLOX-VSMC) by AAV-PCSK9-D374Y injection and high-fat feeding.* ***Results and discussion:*** *Calcified aortic valves show higher LOX expression localized near calcified area, colocalizing to positive cells for RUNX2 and 8-oxo-deoxyguanosine (an oxidative stress marker). VICs exposed to OM exhibited an exacerbated ECM mineralization and an increased ROS production. In these cells, BAPN attenuated both OM-induced calcium deposition and ROS production. Conversely, LOX overexpression in VICs aggravated both ECM mineralization and oxidative stress. Atherosclerosis burden and aortic calcification were exacerbated in atherosclerosis-challenged TgLOX-VSMC mice. In atherosclerotic lesions from brachiocephalic arteries, LOX transgenesis resulted in thicker fibrous cap with higher inflammation, calcification and oxidative stress. TgLOX-VSMC mice also showed increased aortic calcification and oxidative stress in aortic roots.* ***Conclusions:*** *Our findings highlight the key role of LOX activity in cardiovascular calcification and suggest the contribution of LOX-derived ROS to this process. Funded by PID2021-122509OB-I00 (MCIN).*

6. ***Author: Matteo Lemoli[1];*** Co-Authors: Claudia Agabiti Rosei[1], Paolo Malerba[1], Giulia Chiarini[1], Matteo Nardin[1], Francesca Famà[1], Gianluca Edoardo Mario Boari[2], Mattia Baresi[1], Francesca Salvotti[1], Chiara Cattaneo[1], Nicola Laera[1], Cristina Bevacqua[1], Stefano Bressa[1], Gerta Sinaimeri[1], Enzo Porteri[1], Anna Paini[1], Massimo Salvetti[1], Damiano Rizzoni[1,2], Maria Lorenza Muiesan[1], Carolina De Ciuceis[1] Affiliation(s): [1]Internal Medicine, Department of Clinical and Experimental Sciences, University of Brescia; [2]Division of Medicine, Spedali Civili di Brescia, Montichiari Hospital, Brescia, Italy. ***Title:*** *Microcirculation and SARS-CoV2 infection: a follow up study.* ***Background:*** *SARS-CoV2 infection leads*

to several clinical scenarios, named COVID-19. A microcirculation impairment seems to play a key role in the pathophysiology and clinical consequences of COVID-19 but to date there are no evidences of structural microvascular damage in these patients. ***Aim:*** *The aim of this study is to investigate microvascular alterations using adaptive optics and video-capillaroscopy in patients admitted for COVID-19 and re-evaluated one year later.* ***Methods:*** *We enrolled 153 patients (61 ± 11years, 69% males) admitted to the Hospital of Montichiari (Brescia) and to the Internal Medicine Department of ASST Spedali Civili - University of Brescia for respiratory failure due to SARS-CoV2 interstitial pneumonia. Patients were evaluated three months after hospitalization and after one year. All patients underwent hematochemical tests, evaluation of retinal arteriolar morphology by adaptive optics, assess-ment by videocapillaroscopy of fourth finger's basal and total capillary density (BCD and TCD respectively). Results: 87 patients with completed follow-up were analyzed. After one year we observed a reduction of wall to lumen ratio and wall cross sectional area, an increase in internal lumen, a reduction in TCD. No significant differences in BCD were found. Microvascular changes were independent of body mass index and the presence of hypertension or diabetes mellitus.* ***Conclusions:*** *Our data show that patients with SARS-Cov2 infection present an improvement of microvascular structure after one year from the disease, such as a reduction in WLR and WCSA of retinal arterioles. COVID19 might induce microcirculation's structural alterations contributing to vascular damage.*

7. ***Author: Zoe González-Carnicero;*** Co-Authors: Ángela Martín, Raquel Hernanz, María Teresa Barrús, Marta Martínez-Casales, Maria Jesús Alonso. Affiliation(s): Depto. de Ciencias Básicas de la Salud, Facultad de Ciencias de la Salud, Universidad Rey Juan Carlos, Madrid, Spain. ***Title:*** *TLR4 is involved in IL-1β-induced effects. Role of Nrf2. Chronic vascular inflammation, generally associated with oxidative stress, is a key feature of cardiovascular diseases. The proinflammatory cytokine IL-1β (interleukin-1β) contributes to vascular smooth muscle cell (VSMC) phenotypic modulation, vascular remodeling and endothelial dysfunction associated with these pathologies. On the other hand, TLR4 (Toll like receptor 4) activation also contributes to inflammation and vascular alterations in several cardiovascular diseases. Recently, a possible relationship between IL-1β and TLR4 has gained importance. Among the mechanisms that regulate reactive oxygen species (ROS) levels, Nrf2 (nuclear factor erythroid 2-related factor 2), a transcription factor sensitive to oxidative stress that regulates the transcription of antioxidant and anti-inflammatory genes, stands out. Here we analyzed whether TLR4 participates in the IL-1β-induced effects and the role of Nrf2 in such effects. For this, VSMC and mouse aortic segments stimulated with IL-1β were used. In VSMC, IL-1β increases ROS production, expression of proinflammatory MyD88/MAPK/AP-1/AP-1/COX-2 pathway and cell migration and proliferation. Additionally, IL-1β induces endothelial dysfunction in aorta. Furthermore, IL-1β activates Nrf2 pathway, an effect that is reversed by the antioxidant apocynin. Nrf2 Inhibition by using brusatol enhances IL-1β-induced MAPK induction and cell migration. Moreover, the TLR4 inhibitor CLI-095 reduces the pro-oxidant and pro-inflammatory effects of IL-1β, its effect on endothelial function as well as the Nrf2 activation. These results indicate that TLR4/MyD88 pathway is involved in IL-1β-induced pro-oxidant and pro-inflammatory effects. Moreover, IL-1β, through oxidative stress production and TLR4 activation, activates Nrf2, which acts as a compensatory mechanism against the deleterious effects of the cytokine.*

8. ***Author: Alice Bongrani;*** Co-Authors: Tedeschi Stefano, Pilotti Elisabetta, Verzicco Ignazio, Poli Diana, Cabassi Aderville. Affiliation(s): Unità di Ricerca Cardio-Renale e Ipertensione Arteriosa, Unità Operativa Complessa Clinica e Terapia Medica/Dipartimento di Medicina e Chirurgia/Università degli Studi di Parma. ***Title:*** *The lipolytic effect of zinc-alfa2-glycoprotein (ZAG) is related to its antioxidant activity: possible influence of glycosilation pattern.* ***Background:*** *zinc-alfa2-glycoprotein (ZAG) is a 42-KDa lipolytic adipocytokine overexpressed in conditions associated with body wasting, including congestive heart failure (CHF). Beyond β-adrenergic system activation, recent evidence suggests that ZAG involvement in cardiac cachexia (CXC) development also occurs through its action as an AOC3 amino oxidase allosteric inhibitor.* ***Objective:*** *To evaluate ZAG circulating levels in normoxic and hypoxic CHF, as well as CXC, and to assess H2O2 release in primary adipocyte cultures treated with recombinant ZAG or plasma samples from CHF, CXC and Control subjects.* ***Methods:*** *Neurohormonal*

activation and lipolysis markers, as well as ZAG expression in immunoblotting (n=4), were studied in plasma. H2O2 release from isolated adipocytes, obtained by subcutaneous adipose tissue biopsy, was quantified by Amplex Red (n=13). RESULTS: Under hypoxic and CXC conditions, the Western Blot signal of ZAG was simplified from two to a single band, possibly related to changes in its glycosylation pattern. Furthermore, H2O2 release from isolated adipocytes, which was higher in CXC than in CHF and Control cultures, was significantly reduced by recombinant ZAG (25 µg/mL, p<0.05). The same antioxidant effect was obtained by incubating healthy adipocytes with plasma from CXC patients (p<0.001) and was abolished by plasma pre-treatment with an anti-ZAG antibody (p<0.01). ***Conclusions:*** *ZAG has a significant antioxidant effect in human adipocytes, strongly dependent on glycosylation pattern. Particularly, hypoxia and CXC would be associated with an increased ratio of low-to-high glycosylated forms of ZAG, resulting in changes in its antioxidant activity that appear to play a key pathogenetic role in CHF-associated lipolysis.*

9. ***Author: Luigi Marzano [1,2];*** *Co-*Author: ***Claudio Ronco [3,4].*** Affiliation(s): [1]Centro per lo Studio e la Cura dell'Ipertensione Arteriosa, Internal Medicine Unit, San Bortolo Hospital, U.L.S.S. 8 Berica, Vicenza, Italy; [2]Internal Medicine Unit, San Bortolo Hospital, U.L.S.S. 8 Berica, 36100 Vicenza, Italy; [3]Department of Medicine (DIMED), University of Padova - 35128 Padova, Italy; [4]Department of Nephrology, Dialysis and Transplantation, International Renal Research Institute of Vicenza (IRRIV), San Bortolo Hospital - 36100 Vicenza, Italy. ***Title:*** *SOPRANO Study Unveils Clinical and Biochemical Outcomes Post-Adrenalectomy for Primary Aldosteronism in Reference Centers.* ***Background:*** *The uncertainty surrounding hypertension cure following adrenalectomy in unilateral primary aldosteronism (PA) has been a topic of much debate. Our study, part of the Surgical Outcome of PRimary Aldosteronism progNostic mOdels (SOPRANO) initiative,1 aimed to shed light on this issue by analyzing surgical outcomes from tertiary and quaternary referral centers.* ***Methods:*** *We meticulously reviewed 32 studies from 27 publications, focusing on those with low risk of bias. These studies encompassed a diverse patient population from 132 referral centers worldwide, providing a comprehensive view of the clinical landscape.* ***Results:*** *Our analysis included 4,861 patients out of the 5,887 who underwent adrenalectomy. We found that complete clinical and biochemical success rates were 39% (95% CI: 34–44%) and 99% (95% CI: 96–99%) respectively, figures that held steady even when considering only low-bias studies. Interestingly, our multivariate meta-regression analyses revealed that factors such as Body Mass Index (BMI), recruitment time period, and duration of hypertension inversely correlated with complete clinical success. Similarly, BMI and the number of enrolled centers inversely correlated with complete biochemical success.* ***Discussion In conclusion:*** *our study provides robust estimates of success rates following adrenalectomy for unilateral PA. Furthermore, it identifies potential effect modifiers that could help clinicians better inform patients about post-surgery expectations, thereby enhancing treatment effectiveness and patient outcomes. 1 Marzano L, Kazory A, Husain-Syed F, Ronco C. Prognostic Models to Predict Complete Resolution of Hypertension After Adrenalectomy in Primary Aldosteronism: A Systematic Review and Meta-analysis. Clin Endocrinol (Oxf). 2023. doi: 10.1111/cen.14918.*

10. ***Author: Maria Fernanda Fussi;*** Co-Authors: T. Rivabella-Maknis[3], R.P. Bulacio[1,2], M.H. Hazelhoff[1], S.B Marquez[4], R. Campagno[1], J.L. Molinas[5], A. Brandoni[1,2], L.A. Monasterolo[1,2], M.C. Larocca[3], S.M. Molinas[1,2,3]. Affiliation(s): [1]Pharmacology. Faculty of Biochemistry and Pharmacy Sciences. National University of Rosario (UNR), Argentina; [2]National Council for Scientific and Technical Research (CONICET); [3]Institute of Experimental Physiology (IFISE-CONICET); [4]Anatomy and Pathological Physiology. Faculty of Medical Sciences, UNR. 5Human Physiology. Faculty of Medical Sciences, UNR. ***Title:*** *Sex differences in the response to ischemic acute kidney injury and c21 nephroprotection.* ***Background:*** *Animal studies have shown that female sex protects against the development of renal ischemia-reperfusion (IR) injury. We previously demonstrated that AT2R activation by its agonist C21 (1 mg/kg/d), prevented tubular cell damage induced by IR in male rats. This has not been studied in female rats yet.* ***Aims:*** *Our aims were to evaluate sex differences in renal response to IR and compare the effects of C21 pretreatment.* ***Methods:*** *Wistar male (MIR) and female rats (FIR) (n=6 per group) were submitted to 40 min of unilateral renal ischemia followed by 24 h of reperfusion. C21 (0.3*

mg/Kg/d i.p.) was administered for two days prior to IR in male (MIRC21) and female rats (FIRC21). Controls underwent sham operation (MC, FC). Functional and histological studies were performed. Results: Glomerular filtration rate (GFR) estimated by creatinine clearance, was reduced in MIR (-48%) that was not prevented in MIRC21 (-32%). Uremia was increased in MIR (+69%*) that was not prevented in MIRC21 (+81%*). GFR and uremia were not statistically significant in the female group Two way ANOVA-Tukey, *p<0.05 vs MC. Renal cortex histopathological evaluation revealed extensive acute tubular necrosis in MIR, while the damage in MIRC21 was moderate. The tissue damage was moderate in FIR and was mild to normal in FIRC21. Conclusions: We found that IR damage in female rats was less severe than in males. We demonstrated C21 nephroprotective effects against IR tissue damage in female rats.*

11. ***Author: Martina Cebova[1];*** Co-Authors: Andrej Barta[2], Olga Pechanova[3]. Affiliation(s): Institute of Normal and Pathological Physiology Centre of Experimental Medicine Slovak Academy of Sciences. ***Title:*** *Effect of the Nitric Oxide Donor Nicorandil on Biochemical Parameters After Experimentally Induced Myocardial Infarction* ***Background:*** *Myocardial infarction (MI) remains the leading cause of death. Hypertension worsens the prognosis of MI. Nitric oxide (NO) decreases blood pressure and protects the heart at different levels.* ***Aim:*** *to investigate the effect of Nicorandil, NO donor, on biochemical parameters after experimentally induced MI in normal and hypertensive conditions.* ***Methods:*** *To induce MI, the left descending coronary artery was ligated in 2 groups of 16-week-old WKY rats. In one group, NO production was inhibited by L-NAME (20mg/kg/day) administration 4 weeks prior to ligation and NO donor was administered at a dose of 4mg/kg to myocardium prior the reperfusion. Sham operations were performed as controls. 7 days after MI, we evaluated levels of NO-synthase (NOS) activity, eNOS and iNOS protein expressions in ischemic, injured and non-ischemic zones of the heart.* ***Results:*** *MI increased NOS activity in L-NAME group. Nicorandil highlighted this increasing even more. eNOS expression was increased in the injured zone only after MI of L-NAME animals. Conversely, iNOS expression increased in the infarcted zone may contribute to inflammatory process and irreversible necrotic changes. NO donor significantly increased NOS activity/eNOS expression in normotensive groups.* ***Conclusion:*** *Although MI and increased vascular reperfusion of the heart may lead to an inflammatory process and irreversible necrotic changes, persistent elevated level of NOS expression/activity may act as a compensatory mechanism for improvement of myocardial perfusion and this could prevent further cardiac dysfunction and heart failure development. Our results clarify the potential effects of exogenously delivered NO donor during MI. Supported by: VEGA 2/0132/20,APVV-22-0271.*

12. ***Author: Muhammad Saad Salman;*** Co-Authors: Muhammad Salman, Alun Hughes, Jane Maddock, Sarah James, Oli Thomas. Affiliation(s): University College London. ***Title:*** *Dietary sodium over the adult lifecourse and cardiac remodelling. Background: Dietary sodium can influence multiple cardiovascular (CV) conditions, most notably blood pressure (BP). We investigated the relationship of dietary sodium over the adult life course with CV phenotypes.* ***Aims:*** *To investigate the relationship of dietary sodium with CV phenotypes over the adult life course. Methods: Data were drawn from the NSHD population-based British birth cohort (n=832, 52% female) who underwent echocardiography and detailed CV phenotyping at age 60-4. Dietary sodium intake (dNa) was measured using a 5-day diet diary at ages 36, 43, 53 and 60-4. Multivariable linear regression analyses examined associations between dNa and CV phenotypes stratified by sex.* ***Results:*** *In fully adjusted models (accounting for sex, social class, education), dNa was associated with higher LVM indexed to height1.7 (LVMi) at age 60-64 (β= 4.82 [2.63, 7.01]gLVMi/gdNA;p<0.01). This relationship was stronger in women (6.07[3.37, 8.77] gLVMi/gdNA;p<0.01) than in men (3.63g[0.03,7.21] gLVMi/gdNA;p<0.01). Adjusting for systolic and diastolic BP as possible mediators had minimal effects. There was no evidence of an association between sodium and other cardiac and vascular phenotypes.* ***Conclusion:*** *Elevated dietary salt intake across the adult lifecourse is associated with increased LVMi at 60-64y. This association is stronger in females compared with males and is independent of BP. Dietary sodium may be a modifiable risk factor for cardiac remodelling.*

13. ***Author: Raquel Delgado Sarafian;*** Co-Authors AC. Montezano, FJ Rios, L. De Lucca Camargo, R. Touyz. Affiliation(s): [1]Research Institute of the McGill University Health Centre, Montreal, Canada; [2]University of Sao Paulo, Sao Paulo, Brazil. ***Title:*** *Putative role of Notch3 in Brown and White Adipose Tissue. Obesity is associated with cardiovascular disease risk and mechanisms of obesity-induced CVD injury involve adipose tissue dysfunction. Brown adipose tissue is important to thermoregulation and has protective actions in CVDs, while white adipose tissue, when dysfunctional, can induce vascular dysfunction and inflammation. Proteomics analysis of plasma from healthy subjects suggests an association between Notch3 and adiponectin levels. Here, we assessed whether Notch3 is differentially involved in BAT and WAT biology. In this study, gene expression of BAT markers (PRDM16, OTOP1, UCP-1), Notch receptors and downstream targets (Hes1, HeyL, NRIP2) and adiponectin were measured by qPCR, while levels of IL-6 and TNFα were assessed by ELISA in BAT and WAT from female mice with Notch3 overexpression (Notch3) and a gain-of-function mutation vs. WT mice. In BAT, Notch3 overexpression increased gene levels of Hes1 (WT: 1.14±0.2 vs. Notch3: 2.89±0.4-fold change), adiponectin (WT:1.2±0.2 vs. Notch3: 2.9±0.3-fold change), p<0.05, while no changes in BAT markers or cytokines were observed. In WAT, Notch3 overexpression increased gene levels of PRDM16 (WT: 1.18±0.3 vs. Notch3: 3.3±0.7-fold change), UCP-1 (WT:0.6±0.19vs.Notch3:30.18±11.3-fold change),OTOP-1 (WT:2.7±2.1 vs. Notch3: 26.87±10.2-fold change) and IL-6 levels (WT:28.6±3.5 vs. Notch3:48.8±7.8 pg/mg of tissue), p<0.05, while no changes were observed in Notch3 downstream targets or adiponectin, suggesting a browning effect of Notch3 independent of its canonical signaling. Notch3 GOF increased mRNA levels of HeyL, Hes1 and levels of IL-6 only in BAT, with no major effects in WAT.* ***In conclusions:*** *Notch3 may be an important regulator of adiponectin in BAT and possibly in browning of WAT in females.*

14. ***Author: Paloma Palma Guzmán[1];*** Co-Authors: Esther Durán-Mateos [2], Gema Ruíz-Hurtado [3], Elisa Mercado-García [3], Reinhold Kreutz [4], Marta Sanz-Gómez [1], María S. Fernández-Alfonso [1]. Affiliation(s): [1]Instituto Pluridisciplinar and Facultad de Farmacia UCM, Madrid, Spain; [2]Department of Basic Medical Sciences, Universidad San Pablo CEU, Madrid, Spain; [3]Cardiorenal Translational Laboratory, Institute of Research Imas12, Hospital Universitario 12 de Octubre, Madrid, Spain; [4]Charité-Universitätsmedizin Berlin and Berlin Institute of Health, Department of Clinical Pharmacology and Toxicology, Germany. ***Title:*** *Mineralocorticoid receptor antagonism reduces kidney damage and metalloproteinases MMP-2 and MMP-9 activities in diabetic rats with chronic kidney disease.* ***Background:*** *Metalloproteinases (MMP)-2 and MMP-9 are recognised as the main contributors to extracellular matrix degradation. An increase in their activity is thus a risk factor for chronic kidney disease.* ***Aim:*** *To characterize whether treatment with eplerenone (EP), a mineralocorticoid receptor antagonist, induces a decrease in kidney damage through the downregulation of renal MMP-2 and MMP-9 activities in diabetic Munich Wistar Frömter (MWF-D) rats.* ***Methods:*** *Diabetes was induced by a streptozotocin injection (15 mg/Kg, i.p.) together with exposure to a high fat/high sucrose (HF/HS) diet (MWF-D) for 6 weeks. The MWF-D-EP group was additionally treated with 100 mg/Kg/day of EP included in the HF/HS diet (n = 6/group). Results and discussion: MWF-D and MWF-D-EP rats exhibited significantly elevated glycemia, water intake, urine volume, glucosuria, and kidney weight compared to control MWF. Histological examination of kidney samples with hematoxylin and eosin staining showed renal histological abnormalities in MWF, such as widening of Bowmann's space, glomerulosclerosis, interstitial hemorrhage, and mononuclear infiltrated cells between renal tubules. MWF-D presented hyaline cast of renal tubules and tubules droplets, mesangial cell expansion, with thickening of the basement membrane and increased mesangial matrix. MWF-D rats exhibited an increase of kidney damage markers Ngal and Kim-1 which was significantly supressed by EP. Accordingly, MMP-2 and MMP-9 activities determined by zymography were significantly higher in MWF-D compared to MWF and decreased by EP treatment.* ***Conclusion:*** *The improvement of renal damage may be due to a possible effect of EP on MMP-2 and MMP-9 activities regardless of changes in glycemic parameters.*

15. ***Author: Raisa Brito Santos[1,3,4];*** Co-Authors: Gabriel Estrela, Alexandre Gabriel Estrela[2], Alexandre Budu[3], Adriano Arruda[1,3], Ronaldo Araujo[3,4]. Affiliation(s): [1]Federal University of São Paulo Unifesp,

Nephrology, São Paulo, Brazil; [2] National Institutes of Health (NIH); [3]Federal University of São Paulo Unifesp, Biophysics, São Paulo, Brazil; [4]Max Delbrück Center for Molecular Medicine. ***Title:*** *Voluntary exercise exacerbates the fibrosis in the kidney induced by folic acid in male C57BL/6J mice.* ***Background and aims:*** *Voluntary exercise is of utmost importance to avert a plethora of diseases and to ameliorate other conditions, such as neuromuscular diseases, however in some occasions, the kidney inflammation can be exacerbated by intense physical activity. The folic acid kidney disease model is of key interest since it is non-invasive and leads to conspicuous fibrosis. Our main objective was to determine the influence of voluntary exercise on chronic kidney disease in folic acid-treated mice.* ***Methods:*** *Male C57/Bl6 mice were divided into 3 groups, vehicle (V), folic acid (FA), and folic acid + voluntary exercise (FA + E). The animals were kept in cages with or without controlled voluntary wheels (sedentary or exercised groups) for 30 days and then received a single injection of folic (240 mg/kg i.p.) in 0.3 mol/L sodium bicarbonate. The animals were euthanized after 28 days. Results: On day 28 after folic acid administration, the urea levels and creatinine were higher in animals that went in the wheel as well as renal injury markers such as NGAL. Inflammation markers such as IL-6, and IL18, apoptosis markers such as caspase 1, and collagen III expression were exacerbated in animals that went in the wheel. Picrosirius red staining was used to assess tubulointerstitial fibrosis and the kidney architecture; animals from the FA+E group showed the highest destruction of kidney architecture and higher fibrosis score.* ***Conclusion:*** *We established that previous voluntary exercise increases kidney fibrosis on nephrotoxic folic acid – induced model.*

16. ***Author: Rita Ribeiro-Oliveira[1,2];*** Co-Authors: P. Rodríguez-Rodríguez[3], J. B. Sousa[1], I. M. P. L. V. O. Ferreira[2], S. M. Arribas[3] and C. Diniz[1]. Affiliation(s): [1]LAQV/REQUIMTE, Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal; [2]LAQV/REQUIMTE, Laboratory of Bromatology and Hydrology, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal; [3]Department of Physiology, Faculty of Medicine, Universidad Autónoma de Madrid, Madrid, Spain. ***Title:*** *Angiotensin-converting enzyme inhibitory activity elicited by brewing peptides: influence of the oral route and vascular microenvironment.* ***Background:*** *Peptides derived from brewing by-products, namely brewer's spent grain (BSG) and yeast, and a 50:50 mixture of both (MIX), have proven to be good angiotensin-converting enzyme (ACE) inhibitors by in vitro assays. Accordingly, they can prevent the conversion of angiotensin (Ang) I into the vasopressor Ang II having the potential of managing hypertension. However, the influence of the oral route and/or vascular microenvironment on their potential efficacy in inhibiting endogenous ACE remains to be verified. Aim: The impact on vascular response of the ACE-inhibitory activity of BSG and MIX peptides before (BSGI, MIXI) and after (BSGF, MIXF) in vitro simulated oral administration (gastrointestinal digestion, intestinal absorption, and liver metabolism) will be evaluated in hypertensive arteries Methods: Isometric tension recording was employed to examine the effect of MIX/BSG (0.87 mg of protein/mL) or captopril, on vascular ACE in iliac arteries from adult spontaneously hypertensive rats through cumulative curves to ACE substrate (Ang I, 10-9-10-6M). Results and Discussion: The peptides' bioactivity increased after simulated oral administration. While all BSG and MIX peptides exhibited ACE-inhibitory capacity in vitro, only BSGF promoted a significant reduction in vasocontraction induced by the generated Ang II in ex vivo assays, an effect comparable to captopril. BSGF displayed a mixed inhibitory mechanism as evidenced by Michaelis-Menten and Lineweaver-Burk plots.* ***Conclusions:*** *The efficacy of BSG and MIX peptides as ACE inhibitors was influenced by the oral route and vascular microenvironment. BSG seems to offer potential benefits in the management of hypertension with an equivalent efficacy/potency as captopril.*

17. ***Author: S. Ruvira;*** Co-Authors: P. Rodríguez-Rodríguez, F. Abderrahim, M. Iampanichakul, D Ramiro-Cortijo, and S.M. Arribas. Affiliation(s): Department of Physiology, Faculty of Medicine. Universidad Autónoma de Madrid (Spain); Department of Physiology, Faculty of Medicine. Khon Kaen University (Thailand). ***Title:*** *Alterations in mesenteric resistance artery function in females exposed to fetal undernutrition.* ***Background:*** *Exposure to stress factors during intrauterine life programs the fetus and predisposes to cardiovascular diseases in adult life. In rats exposed to fetal undernutrition due to*

maternal nutrient restriction in gestation (MUN), males develop hypertension at the age of 6 months, while females remain normotensive in adult age, but show higher blood pressure levels in ageing (Gutiérrez-Arzapalo et al., Biomedicines. 2020; 8(10):424). Aims: Our aim is to evaluate if MUN females have alterations in resistance artery function in adult life, which may contribute to hypertension development in ageing. Methods. Eight-month-old female rats (MUN, exposed to fetal undernutrition, n=5; Control, n=6) were used. Mesenteric resistance artery function was studied with wire myography, assessing KCl, noradrenaline (NA), acetylcholine (ACh), and sodium nitroprusside (SNP) concentration-response curves. The contribution of cGMP-dependent gasotransmitters (NO, CO, and H2S), prostanoids, and EDHF were evaluated by preincubation with L-NAME, ODQ or and indomethacin prior to acetylcholine concentration-response curve. ***Results:*** *No differences in KCl, but significantly larger maximal NA contraction in MUN rats compared to control (p=0.04). ACh relaxations and PD2 tended to be smaller in MUN (p=0.09). Indomethacin increased ACh vasodilatation, to a similar extent in MUN and Control. The contribution of NO and other gasotransmitters to ACh relaxation was similar in MUN and control rats; but EDHF contribution tended to be smaller in MUN (0.063). No differences in SNP responses were found.* ***Conclusions:*** *Increased NA responses and reduced EDHF vasodilatation in resistance arteries may contribute to the development of hypertension in MUN females in aging.*

18. ***Author: Safiya Abdi Shugri Ahmed[1];*** Co-Authors: Antonina Nazarova[2], Inesa Uzunjan[1], Colin Sumners[3], Tore Bjerregaard Stage[4], Per Svenningsen[1], Vsevolod Katritch[2], Ulrike Muscha Steckelings[1], Igor Maciel Souza-Silva[1]. Affiliation(s): [1]IMM - Department of Cardiovascular and Renal Research, University of Southern Denmark, Odense, Denmark; [2]Center for New Technologies in Drug Discovery and Development, Bridge Institute, Michelson Center for Convergent Biosciences, University of Southern California, Los Angeles, CA, USA; [3]Department of Physiology and Aging, University of Florida, Gainesville, USA; [4]Department of Public Health, University of Southern Denmark, Odense, Denmark. ***Title:*** *Investigations on the molecular interactions of angiotensin-(1-5) with the angiotensin AT2-receptor for receptor activation.* ***Background:*** *Our group found that angiotensin-(1-5) (Ang-(1-5)) is a biologically active component of the RAS and an AT2R agonist. However, the molecular interaction of Ang-(1-5) with the AT2R leading to AT2R activation is unknown.* ***Aim:*** *To investigate the molecular mechanisms of AT2R activation by Ang-(1-5). Method: In silico docking simulations were performed in order to identify amino acid residues within the AT2R that are involved in Ang-(1-5) binding. The respective amino acids were mutated to Ala by site directed mutagenesis using the primer extension method (PIPE). Chinese hamster ovary cells (CHO) were transfected with the wildtype AT2R or mutated AT2R (Arg185Ala, Asp297Ala or Lys215Ala), stimulated with Ang-(1-5) (concentration ranging from 10-11 M to 10-6 M in a half log scale) for 15 minutes and resulting in nitric oxide (NO) release quantified by fluorescence microscopy. Results: In silico docking simulations confirmed binding of Ang-(1-5) to the AT2R and identified Arg185, Asp297 and Lys215 as important for ligand-receptor interactions. The Asp297Ala and Arg185Ala mutations caused a weaker response to Ang-(1-5) compared to the wildtype receptor, while the Lys215Ala mutation did not alter the response.* ***Conclusion:*** *From our results we conclude that Asp297 and Arg185 within the AT2R are important for the binding and activation of the AT2R by Ang-(1-5). The results further indicate that Ang-(1-5) binds deeply into the receptor pocket in a way different from Ang II binding to the AT2R.*

19. ***Author: Sara Jiménez-González[1];*** Co-Authors: A. Romero-Miranda[1], B. Delgado-Valero[1], B. Ramchandani[2], F. Islas[3], E. Martínez-Martínez[1,4], V. Cachofeiro[1,4]. Affiliation(s): [1]Departamento de Fisiología, Facultad de Medicina, Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM), Universidad Complutense de Madrid, Madrid, Spain; [2]Servicio de Cirugía Cardiaca Infantil, Hospital La Paz, Madrid, Spain; [3]Servicio de Cardiología, Instituto Cardiovascular, Hospital Clínico San Carlos, Madrid, Spain; [4]Ciber de Enfermedades Cardiovasculares (CIBERCV), Instituto de Salud Carlos III, Majadahonda, Spain. ***Title:*** *The interaction between Galectin-3 and endoplasmic reticulum stress mediates the cardiac alterations associated with myocardial infarction in obese rats.* ***Background:*** *Galectin-3 (Gal-3) and endoplasmic reticulum stress (ERS) have been described as possible mechanisms involved in the progression of cardiac damage associated with obesity and myocardial*

infarction (MI). ***Aim(s):*** *To assess the possible interactions of Gal-3 and ERS in the cardiac alterations associated with MI in the context of obesity.* ***Methods:*** *Male Wistar rats were fed a high fat diet (35% fat) for 10 weeks. At the sixth week, when obesity was established, MI was induced (HFD+MI), and different treatments were administered for four weeks: modified citrus pectin (MCP; 100 mg/kg/day; inhibitor of the activity of Gal-3); 4-phenylbutiric acid (4-PBA; 500 mg/kg/day; inhibitor of ERS); or vehicle. Rats fed a standard diet (3.5% fat) and with sham surgery were defined as control group (CT).* ***Results:*** *MCP and 4-PBA improved the alterations in diastolic and systolic functions observed in infarcted-obese animals without affecting the increase in body weight or systolic blood pressure. 4-PBA but not MCP prevented the cardiac hypertrophy observed in HFD+MI. Both treatments reduced the cardiac fibrosis and the increase in extracellular matrix proteins and mediators that could explain the improvements observed. Interestingly, MCP and 4-PBA blocked the increase in cardiac Gal-3 levels and the activation of ERS in HFD+MI animals. Both treatments also prevented cardiac oxidative stress and inflammation in infarcted-obese animals.* ***Conclusions:*** *A crosslink between Gal-3 and ERS could play a relevant role in the development of functional alterations and cardiac fibrosis associated with MI in the context of obesity and emerging as a new therapeutical target.*

20. ***Author: V. López-Miranda[1,2,4];*** Co-Authors: A. González[1,2], S. Flaj[1], V. Fernández-Cabello[3], R. Hernanz[1,5], MJ. Alonso[1,5], E. Quesada[2,3,4], E. Herradón[1,2,4]. Affiliation(s): [1]Dept. Basic Health Sciences, Faculty Health Sciences, University Rey Juan Carlos (URJC); [2]PHARMAKOM High-Performance Group; [3]Institute of Medical Chemistry, Spanish National Research Council (CSIC); [4]Associated R&D Unit of the Institute of Medical Chemistry (IQM), Spanish National Research Council (CSIC); [5]INVASC Consolidated Research Group. ***Title:*** *Effect of TRL4 blockade on cardio-metabolic and renal alterations caused by diet induced metabolic syndrome. Modulation of the TRL4/MYD88/NLRP3 axis expression.* ***Background:*** *TLR4 receptor activation has been involved in cardio-metabolic complications in Metabolic Syndrome (MS), proposing TLR4 receptor blockade as a possible therapeutic strategy. Objectives: 1) to evaluate whether a TLR4 receptor antagonist (TLR4-ANT) could improve cardio-metabolic alterations in an experimental model of MS; 2) to analyze whether changes in the expression of TLR4, MyD88 and NLRP3 in cardiovascular and renal tissues are involved in TLR4 blockade effect.* ***Methods:*** *Adult male Wistar rats were used. Experimental groups (n=8-10): a) Control, animals fed on a standard diet for 20 weeks; b) MS, animals fed on a high-fat hypercaloric diet for 20 weeks; c) MS+TLR4-ANT, animals fed on a high-fat hypercaloric diet for 20 weeks + 10 mg/Kg of TLR4-ANT (i.p.) daily for the last two weeks. In all groups, at the end of the experimental period, we evaluated: feeding behavior, anthropometric and biochemical parameters, blood pressure, heart rate, cardiac function, and vascular reactivity. TLR4, MyD88 and NLRP3 expression was also determined in target tissues by Western Blot. Results and Discussion: TLR4-ANT restored caloric intake, increased body weight, abdominal perimeter, as well as plasma levels of glucose, triglycerides, cholesterol, LDL and creatinine in animals with MS. Besides, TLR4 blockade restored hypotension and endothelial dysfunction in resistance vessels. Furthermore, TLR4-ANT did not modify TLR4 expression, but it changed the expression of MyD88 and NLRP3 at vascular and renal level.* ***Conclusions:*** *Blockade of TLR4 receptor can correct cardio-metabolic and renal alterations in MS, involving changes in the expression of MyD88 and NLRP3.*

21. ***Author: Alejandro Montoro-Garrido[1];*** Co-Authors: M. Cuesta-Corral[1]; A. Romero-Miranda[1]; B. Ramchandani[3]; F. Islas[2]; E. Martínez-Martínez[1,4]; V. Cachofeiro[1,4]. Affiliation(s): [1]Departamento de Fisiología, Facultad de Medicina, Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM), Universidad Complutense de Madrid, 28040 Madrid, España; [2]Servicio de Cardiología, Instituto Cardiovascular, Hospital Clínico San Carlos, 28040 Madrid, España; [3]Servicio de Cirugía Cardiaca Infantil, Hospital La Paz, 28046 Madrid, España; [4]Ciber de Enfermedades Cardiovasculares (CIBERCV), Instituto de Salud Carlos III, 28222 Majadahonda, España. ***Title:*** *The use of mitochondrial transplantation on the management of renal complications associated with cardiorenal syndrome.* ***Background:*** *Cardiorenal syndrome (CRS) is a clinical condition that impacts both the heart and the kidneys. Mitochondrial dysfunction can play an important role in this scenario. Previous studies have shown the benefitial effects of mitochondrial transplantation at cardiac or renal level in acute damage,*

however, there are no studies of mitochondrial transplantation in CRS or in chronic studies. Objective(s) The objective of the study was to evaluate the impact of mitochondrial transplantation at cardiac level in the renal alterations related to myocardial infarction (MI). ***Methods*** *MI was developed in male Wistar rats by the ligation of the left coronary artery. Half of the animals were treated with mitochondrial transplantation in the heart (180 µg in PBS). A group of rats with sham operation was used as reference group. Cardiac function was evaluated 4 weeks after the surgery and previous to the sacrifice. Markers of inflammation, oxidative stress, ER stress and fibrosis were analyzed at renal level. Results and Discussion Mitochondrial transplantation improved cardiac function in MI animals. At renal level, MI animals showed renal damage characterized by an increase in NGAL (a marker of acute kidney injury). This damage was accompanied by an increase in extracellular matrix components, as well as an activation of ER stress, upregulation of superoxide anions and inflammatory markers. The administration of mitochondria in the heart was able to prevent all of these alterations.* ***Conclusions*** *These results suggest that mitochondrial administration at cardiac level could be a therapeutical approach for the renal alterations induced by MI.*