European Council for Cardiovascular Research

2021 ECCR Virtual Meeting
8th – 9th October 2021

Friday, October 8th, 2021 (All times are CEST/CET)

09.00–09.15 Welcome and opening – *Marisol Fernandez-Alfonso, ECCR president*

9.15-11.00 ECRs selected oral communications.

Moderators: Marta Gil Ortega (Spain), Armond Daci (Kosovo)

See attached ECR programme

11.00-11.15 Break

11.15–13.00 Session 1 – Cardiovascular health in women.

Women in Hypertension Research

(Joint session with WiHR – Women in Hypertension Research ISH)

Moderator: Ulrike Muscha Steckelings (Denmark), Maria Christina Zennaro (France)

11.15-11.40 - *Genetics of coronary artery dissection and fibromuscular dysplasia. Nabila Bouatia-Naji (France)*

11.40-12.05 - *Mineralocorticoid receptor blockade and cardiovascular health in women. Antoine Ouvrard Pascaud (France)*

12.05-12.30 - *Age and sex in hypertension prevalence, awareness, and control. Alexandra Konradi (Russia)*

12.30-12.55 *Novel aspects of Preeclampsia. Christian Delles (UK)*

13.00-14.00 Break

14.00-15.45 Session 2 –Vascular damage: an update on mechanisms

Moderator: Damiano Rizzoni (Italy), Carmine Savoia (Italy)

14.00-14.25 - *Complex mechanisms of endothelial dysfunction; in vivo endothelial profiling in murine model of cardiovascular disease. Stefan Chlopicki (Poland)*

14.25-14.50 - *Mechanisms of vascular remodeling and stiffening. Luis Martinez-Lemus (USA)*

14.50-15.15 - *The Nitric Oxide Signalling Pathway in Aortic Aneurysm and Dissection. Juan Miguel Redondo (Spain)*

15.15-15.40 - *Long Non-Coding RNA in vascular disease. Reinier A Boon (The Netherlands)*

15.45-16.00 Break

16.00-17.45 Session 3 – Hot topics: Covid-19 and cardiovascular disease

DZHK DEUTSCHES ZENTRUM FÜR HERZ-KREISLAUF-FORSCHUNG E.V.

Joint session sponsored by DZHK (German Center for Cardiovascular Research)
Moderator Michael Bader (Germany), Marisol Fernández-Alfonso (Spain)

16.00-16.25 - *COVID-19 - an immunothrombotic disease. Ursula Rauch-Kröhnert (Germany)*

16.25-16.50 - *Autoimmune mechanisms in COVID-19 associated coagulopathy. Wolfram Ruf (Germany)*

16.50-17.15 - *The Role of Anti-Coagulation and Beta-blockers in COVID-19 Patients. Valentín Fuster (USA/Spain)*

17.15-17.40 - *The different faces of COVID-19-related heart disease. Sophie Van Linthout (Germany)*

17.45-18.00 Break

18.00-19.20 Session 4 – Early career researchers' session.

Moderators: Giacomo Rossito (Italy), Myrthe Van Der Bruggen (The Netherlands)

18.00-18.25 - *How to avoid the impostor syndrome in Science – Stephanie Watts (USA)*

18.25-18.45 - *How to write a grant application – Jennifer Pollock (USA)*

18.45-19.05 - *How to start a successful early career in research. Paul Meakin (UK).*

19.30-20.30 Social activity. *Hosted by Augusto Montezano*

Saturday, October 9th, 2021 (All times are CEST/CET)

8.30-10.15 ***Poster sessions 1 and 2 + Oral Communications sessions 1 and 2***

See attached Oral/Poster session programme

10.15 – 10.30 Break

10.30-12.15 Session 5 – Novel aspects of immunity in cardiovascular disease

Moderator: Agostino Virdis (Italy), Ana Briones (Spain)

10.30-10.55 - *Neural Control of Immunity in Cardiovascular Diseases. Daniela Carnevale (Italy)*

10.55-11.20 - *B lymphocytes and atherosclerosis. Almudena Ramiro (Spain)*

11.20-11.45 - *The role of sodium in modulating immune cell function. Dominik N. Müller (Germany)*

11.45-12.10 - *Lipid mediators for the resolution of inflammation in atherosclerosis and valvular heart disease. Magnus Bäck (Sweden)*

12.15-12.30 Break

12.30-13.30 Session 6 – Business meets Academia. Science and technology for life.

Moderator: Koen Reesink (Netherlands), Marko Poglitsch (Austria)

Multi-modal (single) cell and tissue phenotyping technology for mechanobiology research – an evolving business perspective. Joint presentation and demos by Confocal.nl – Jeroen Kole and Optics11Life - Jakob Pyszkowski.

13.30-13.45 Awards

13.45-14.00 Closing remarks

Sponsored by

Friday, October 8th, 2021 (All times are CEST/CET)

9.15-11.00 ECRs selected oral communications.

09.15-09.30 - *Isgylation is involved in aneurysms formation in hypertension.*
María González Amor, AB. García-Redondo, C. Rodriguez, MJ. Ruiz-Rodríguez, R. Rodrigues-Díez, J. Martínez-González, JM. Redondo, S. Guerra, M. Salaices, AM Briones.

09.30-09.45 - *Agtr1a receptors in the subfornical organ activate adaptive immunity in the spleen in response to hypertensive stimuli, promoting blood pressure increase and target organ.*
Marialuisa Perrotta, Pallante Fabio, Carnevale Lorenzo, Mastroiacovo Francesco, Migliaccio Agnese, Cifelli Giuseppe, Lembo Giuseppe, Carnevale Daniela.

09.45-10.00 - *Molecular Mechanisms Involved in the Vascular Protective Effects of Mas1:ETBR Interaction.*
Jithin Kuriakose, A.C. Montezano, R. A. Lopes, Angie YY Sin, Delyth Graham, G. S. Baillie, R. M. Touyz.

10.00-10.15 - *Development of an adrenocortical cell model of calcium balance modulation to decipher the molecular consequences of mutations responsible for primary aldosteronism.*
Bakhta FedLaoui, B. Fedlaoui, T. Cosentino, I. Giscos-Douriez, L. Borowski, FL. Fernandes-Rosa, C. Magnus, SE. Sternson, MC. Zennaro, S. Boulkroun

10.15-10.30 - *Immune-metabolome response to a single exercise exertion reveals dysfunctional metabolic recovery in heart failure.*
Krithika Swaminathan, Aycen Koc, Sabine Kaczmarek, Kristin Lehnert, Ines Urbaneck, Ulf Landmesser, Stephan B. Felix, Marcus Dörr, Martin Bahls, Nicolle Kränkel.

10.30-10.45 - *Complement factor D and C3 are associated with arterial stiffness, independent of age, sex, heart rate and blood pressure but not of cardiometabolic factors: The Maastricht Study.*
Shunxin Jin, Koen D Reesink, Abraham A Kroon, Bastiaan de Galan, Carla JH van der Kallen, Anke Wesselius,Casper G Schalkwijk Coen DA Stehouwer,Marleen MJ van Greevenbroek.

10.45-11.00 - *Inhibition of endoplasmic reticulum stress as a therapeutic strategy to limit cardiac hypertrophic remodeling associated to hypertension.*
Júlia Caparrós (Dr Galán will present on behalf of Júlia Caparrós) Juan Antonio Arroyo, Attila Tobor, Laia Cañes, Miquel Navas-Madroñal, Elisabeth Pérez-Chust, José Martínez-González, Cristina Rodriguez, María Galán.

Saturday, October 9th, 2021 (All times are CEST/CET)

Oral Communication Session 1
Moderator: Florian Limbourg, Ana Briones / Host: Dr Augusto Montezano

08.30-08.45 - *Complement factor D and C3 are associated with arterial stiffness, independent of age, sex, heart rate and blood pressure but not of cardiometabolic factors: The Maastricht Study*. **Shunxin Jin** , Koen D Reesink, Abraham A Kroon, Bastiaan de Galan, Carla JH van der Kallen, Anke Wesselius, Casper G Schalkwijk, Coen DA Stehouwer, Marleen MJ van Greevenbroek.

08.45-09.00 - *RvD2 prevents abdominal aortic aneurysm formation and ameliorates vascular function in Angiotensin II-treated obese mice*. **Raquel Rodrigues Díez,** Constanza Ballesteros-Martinez, Rosa Moreno-Carriles, Mercedes Salaices, Ana M. Briones.

09.00-09.15 - *Inhibition of pyruvate dehydrogenase kinase prevents abdominal aortic aneurysm formation in mice.* **Silke Griepke Dam Nielsen,** M. P. Fonseca, E. Grupe, L. B. Steffensen, J. S. Lindholt, J. Stubbe , D. F. J. Ketelhuth.

09.15-09.30 - *PARP/TRPM2 inhibition attenuates VEGF inhibitor-induced vascular dysfunction.* **Karla Bianca Neves**, R. Alves-Lopes, N.N. Lang, A.C. Montezano, R.M. Touyz

09.30-09.45 - *Supplementation of methylglyoxal in drinking water does not affect the cerebral microvasculature and cognitive function in healthy young mice*. **Eline Berends,** Naima Amiri , Marjo PH van de Waarenburg, Jean LJM Scheijen, Denise JHP Hermes, Robert J van Oostenbrugge, Sébastien Foulquier, Casper G Schalkwijk

09.45-10.00 - *The mitochondrial oxidative stress-endoplasmic reticulum stress axis in the development of cardiovascular fibrosis in obese rats.* **Sara Jiménez González,** S. Jiménez-González, F.V. Souza-Neto, B. Delgado-Valero, R. Jurado-López, M. Genty, A. Romero-Miranda, C. Rodríguez, M.L. Nieto, E. Martínez-Martínez, V. Cachofeiro.

10.00-10.15 - *ACE2 is involved in SARS-CoV-2 induced endothelial cell inflammation independent of viral replication.* **Augusto C Montezano,** Livia De Lucca Camargo, Sheon Samji,

Karla Neves, Francisco Rios, Rheure A Lopes, Wendy Beattie, Imogen Herbert, Vanessa Herder, Agnieszka M Szemiel, Steven McFarlane, Massimo Palmarini, David Bhella, Rhian M Touyz.

Oral Communication Session 2
Moderator: Koen Reesink, Maria Christina Zennaro /Host: Dr Francisco Rios.

08.30-08.45 - *Redox activation of PARP/TRPM2 signalling contributes to vascular damage induced by high salt diet without influencing blood pressure.* **Rhéure Alves-Lopes**, Karla B Neves, Augusto C Montezano, Sheon Samji, Christian Delles, Rhian M Touyz.

08.45-09.0 - *mTORC1 activation and mitochondrial dysfunction in diabetic and hypertensive cardiomyopathy.* **Tianyu Hang,** Corrales S., Azkargorta M, Elortza F, Martínez-Chantar M, Egido J and Lorenzo O.

09.00-09.15 - *Pulsatile biaxial mechanical behavior at different levels of smooth muscle tone: A pilot study.* **Msc Koen van der Laan, K.** van der Laan, M. van der Bruggen, A. Juttner, P. Spronck, C. Schalkwijk, A. Roks, T. Delhaas, B. Spronck, K. Reesink.

09.15-09.30 - *Spike protein 1 of SARS-CoV-2 induces inflammatory response in endothelial cells by increasing interferon stimulated responses.* **Francisco J. Rios,** Augusto C Montezano, Livia L Camargo, Rheure A Lopes, Elihu Aranday-Cortes, John McLauchlan, Rhian M Touyz.

09.30-09.45 - *Vascular and hormonal interactions in primary aldosteronism.* **Alaa B Abdellatif,** Isabelle Giscos-Douriez, Fabio Fernandes-Rosa, Yunling Xu, Sheerazed Boulkroun, Maria-Christina Zennaro.

09.45-10.00 - *Resolvin E1 mitigates endothelial senescence induced by doxorubicin via modulating NLRP3 inflammasome activation.* **Licia Shamoon B.Pharm,** J. Espitia-Corredor, P. Dongil, I. Valencia, M. Menendez-Ribes, G. Díaz-Araya, C. Sánchez-Ferrer, C. Peiró.

10.00-10.15 - *Amlodipine prevents microglia activation and cognitive dysfunction in aged hypertensive mice*. **S. Foulquier,** D. Kerkhofs, R. Helgers, H. van Essen, P. Leenders, D. Hermes, He Steinbusch, J. Prickaerts, E.A. Biessen, R.J. van Oostenbrugge, S. Foulquier.

Poster Session 1
Moderator Martina Cebova, María Bloksgaard/Host Dr Giacomo Rossitto

08.30-08.38 - *ACE-2 expression is modulated by AT1R signaling in human bronchial epithelial cells.* **Ilaria Caputo,** B. Caroccia, T. M. Seccia, G.P. Rossi.

08.38-08.46 - *3D histology of cerebral aneurysms reveals spatial reorganization of the smooth muscle cells in the aneurysmal wall.* **Dr. Maria Bloksgaard,** K. Rosenstand, I. Nissen, P. S. Jensen, S. Munthe, T. H. Nielsen.

08.46-08.52 - *A high-throughput nitric oxide measurement assay to evaluate AT2 receptor activation in vitro reveals that angiotensin-(1-5) is an AT2 receptor agonist.* **Igor Maciel Souza-Silva,** Kenneth Kjærgaard, Christina Mortensen, Tore Bjerregaard Stage, Ulrike Muscha Steckelings.

08.52-09.00 - *Association of ACE2 polymorphisms with obesity and elevated lipids in female adolescents.* **Jairo Lumpuy-Castillo**, Claudia Vales-Villamarín Fernández, Iris Pérez Nadador, Leandro Soriano Guillén, Óscar Lorenzo González, Carmen Garcés Segura.

09.00-09.08 - *Cardioprotective effects of AT2 and Mas receptor activation in angiotensin II dependent hypertension.* **Giovanna Castoldi,** G. Castoldi, R. Carletti, Silvia Ippolito, Andrea Stella, Gianpaolo Zerbini, Giovanni Zatti, Cira RT di Gioia.

09.08-09.16 - *Comparison between first and second/third wave in moderate to severe COVID-19 in a hospital setting in Lombardy (Italy).* **Andrea Corbani,** G.E.M. Boari; F. Leidi; B. Accordini; F. Napoli; C. Ghidelli; G. Archenti; G. Gorla; B. Mangili; O. Scarano; D. Turini; M. Saottini; V. Guarinoni; G. Ferrari-Toninelli; F. Manzoni; D. Rizzoni.

09.16-09.24 - *Differential Contribution of Renal Cytochrome P450 Derivatives to Kidney Endothelial Dysfunction and Vascular Oxidative Stress in Obesity.* **M. Muñoz,** M.E. López-Oliva, E. Pinilla, C. Rodríguez, M.P. Martínez, J. Sáenz-Medina, C. Contreras, A. Sánchez, A. Gómez del Val, L. Rivera, D. Prieto.

09.24-09.32 - *Effect of no donor, co donor and anti-hmgb1 protein in experimental myocardial infarction.* **Martina Cebova,** Andrej Barta, Olga Pechanoa.

09.32-09.40 - *High vascular expression of Lysyl Oxidase (LOX) exacerbates atherosclerosis and vascular calcification.* **Carme Ballester-Servera,** C. Ballester-Servera, L. Cañes, J. Alonso, S. Aguiló, C. Rodríguez, J. Martínez-González.

09.40-09.48 - *Peri-spinal neurovascular response triggered by a painless peripheral stimulus in patients with chronic high blood pressure.* **Juan Pablo González Appelgren,** R. Caulier, JE. Oyarzún1, A. Eblen-Zajjur, S. Uribe.

09.48-09.52 - *Serum dicarbonyl and AGE levels are not associated with levels of AT1AA in patients with aldosterone-producing adenoma.* **Maria Piazza**, N.M.J Hanssen, J Scheijen, M. vd Waarenburg, B. Caroccia, T.M. Seccia, C.D.A. Stehouwer, G.P. Rossi, C.G. Schalkwijk.

09.52-10.00 - *Fat-1 transgenic mice are protected against vascular damage in hypertension.* **Lucía Serrano,** A. B. García-Redondo, M. Salaices, A. M. Briones.

Poster Session 2
Moderator Carmine Savoia, Marko Poglitsch/Host: Margaret Kinninmont

08.30-08.38 - *Correct timing in hospital admission impacts on outcome of COVID-19 elderly patients: a comparison between first and second wave*. **Giulia Gorla, MD**, G.E.M. Boari; O. Scarano; B. Mangili; A. Corbani; F. Leidi; B. Accordini; F. Napoli; C. Ghidelli; G. Archenti; D. Turini; M. Saottini; V. Guarinoni; G. Ferrari-Toninelli; F. Manzoni; A. Marengoni; D. Rizzoni.

08.38-08.46 - *Long-term effect of esmolol on the left ventricle structure in an experimental model of ventricular hypertrophy.* **I. Solchaga-Sánchez,** J.J. Gómez-de Diego, P. Rodríguez-Rodríguez, D. Muñoz, M.J. Delgado, B. Quintana-Villamandos.

08.46-08.52 - *Resolvin E1 attenuates doxorubicin- and interleukin-1beta-induced cardiac fibroblast senescence*. **Jenaro Espitia-Corredor,** L. Shamoon; C. Rimassa-Taré; C. Sánchez-Ferrer; C. Peiró; G. Díaz-Araya.

08.52-09.00 - *Role of Microsomal Prostaglandin E Synthase-1 (mPGES-1) in the metabolic, cardiovascular and renal alterations associated with obesity*. **Constanza Ballesteros-Martinez**, Raquel Rodrigues-Diez, Ernesto Martínez-Martínez, Luis Beltrán, María Gonzalez-Amor, Victoria Cachofeiro, Mercedes Salaices, Ana M. Briones.

09.00-09.08 - *The diabetic munich wistar frömter rat: a new model of diabetic nephropathy*. Elena Vega-Martín, **Daniel González Moreno,** Marta Sanz Gómez, Francisco J. Manzano-Lista, Marta Gil-Ortega, Beatriz Somoza, Reinhold Kreutz, María S. Fernández-Alfonso.

09.08-09.16 - *The impact of Transcatheter Aortic Valve Implantation (TAVI) on the function of resistance arteries in patients with severe aortic stenosis.* **Carlo Barsali,** Carlo Barsali, Armando Ferrera, Alberto Michielon, Gaetano Marino, Massimo Volpe, Carmine Savoia.

09.16-09.24 - *The spiranic compound SPI3 activates the endothelial AMPK-eNOS pathway leading to a nitric oxide-dependent vasodilation.* **Marta Sanz-Gómez,** S. Quesada, L. Lagartera, M. Beroiz, J. Cumella, C. Pérez, A. Castro, MS. Fernández-Alfonso.

09.24-09.32 - *Vasoactive Properties of Cocoa Shell waste-product, a potential nutraceutical for cardiovascular disease*. **Kendal Ragusky,** Pilar Rodriguez-Rodriguez, Sophida Puthong, Santiago Ruvira, Silvia Cañas, Miguel Rebollo-Hernanz, Mº Angeles Martín-Cabrejas and Silvia M. Arribas.

09.32-09.40 - *Hemodynamic phenotyping of transgenic rats with ubiquitous expression of an angiotensin-(1-7)-producing fusion protein.* **Daniele Teixeira Alves**, L. F. Mendes; W. O. Sampaio; L. M. C. C. Campos; M. A. R. Vieira; A J. Ferreira; A. S. Martins; E. Popova; M. Todiras; F. Qadri; N. Alenina; M. Bader; R. A. S. Santos; M. J. Campagnole-Santos.

09.40-09.48 - *Prospective Biobank for Ascending Thoracic Aortic Aneurysm Research: Integrating Clinical, Imaging and Pathophysiology Perspectives*. **Berta Ganizada,** S. Parikh, M. Ramaekers, A.C. Akbulut, I. Cortenraad, F. Kerckhove, C. Willems, G. Debeij, E. Natour, R. Lorusso, J. Wildberger, J.G. Maessen, R. Accord, S. Schalla, K. Reesink, L. Schurgers, E. Bidar.

09.48-09.52 - *In Silico Modeling for Tissue Engineered Heart Repair*. **Yong Wang,** M. Kalhöfer-Köchling, Y.X. Zhang, M. Uecker, S. Boretius, W.-H. Zimmermann, E. Bodenschatz.

09.52-10.00 - *Advanced brain injury characterization by neuroimaging in a mouse model of hypertension-induced cognitive decline*. **Lorenzo Carnevale,** Raimondo Carnevale, Francesco Mastroiacovo, Marialuisa Perrotta, Daniela Carnevale, Giuseppe Lembo.

Full list of abstracts in order of programme.

ECRs selected oral communications.

1. **Author: María González-Amor;** Co Authors: AB. García-Redondo, C. Rodriguez, MJ. Ruiz-Rodríguez, R. Rodrigues-Díez, J. Martínez-González, JM. Redondo, S. Guerra, M. Salaices, AM Briones. Affiliations: Dpto. Farmacología, Facultad de Medicina, Universidad Autónoma de Madrid, Instituto de Investigacion La Paz, Madrid, CiberCV, Spain. ***Title:*** *Isgylation is involved in aneurysms formation in hypertension. Background. Vascular remodelling and altered vascular mechanics are common features of hypertension and are involved in aneurysms development. During last years, it became evident the importance of low-grade inflammation in the vascular alterations associated with hypertension. Earlier studies identified interferon-γ (IFNγ) as an important cytokine involved in vascular damage in hypertension. Interferon Stimulated Gene (ISG) 15 protein can be secreted as a free form and it also produces a post-translational modification (ISGylation), which is reversible by USP18 which is the only enzyme able to separate ISG15 from the substrate protein. ISG15 works as antiviral molecule but the role of ISG15/USP18 in the cardiovascular system is very unknown. Aim: we evaluated the role of ISG15/USP18 pathway in aneurysms formation and possible underlying mechanisms. Methods. We measured ISG15 y murine and human aneurysms. We used wild type mice and USP18C61A mice, which present excessive ISGylation, infused with AngII (1.44mg/Kg/day, 14 days). We measured systolic blood pressure and performed in vivo ultrasound imaging to study aortas' diameter. We also analysed vascular remodelling and mechanics. Finally, the implication of oxidative stress was also studied. Results. Human and murine aneurysms shown enhanced ISG15 expression. USP18C61A mice showed increased AngII-induced mortality, aortic dilation, hypertension and vascular remodelling along with elastin breaks. ISGylation also increases inflammatory markers and reactive oxygen species (ROS) generation. ROS blockade decreased ISGylation-associated increased blood pressure, mortality and aneurysms development. Conclusion: ISG15/USP18 pathway is a novel mediator involved in aneurysms development through increased oxidative stress.*

2. **Author: Perrotta Marialuisa,** Co-Authors: Pallante Fabio, Carnevale Lorenzo, Mastroiacovo Francesco, Migliaccio Agnese, Cifelli Giuseppe, Lembo Giuseppe, Carnevale Daniela. Affiliations: Department of Molecular Medicine, Sapienza University of Rome, Italy; Dipartment of Angiocardioneurology and Translational Medicine, IRCCS Neuromed, Pozzilli, Italy. ***Title****: Agtr1a receptors in the subfornical organ activate adaptive immunity in the spleen in response to hypertensive stimuli, promoting blood pressure increase and target organ. The subfornical organs (SFO) is a brain region with a leaky blood brain barrier exposed to circulating AngiotensinII (AngII). AngII type 1a receptors (AT1aR) are densely expressed in the SFO. We showed that chronic AngII increases blood pressure (BP) by recruiting adaptive immune response in mice. Whether the splenic immune response is under brain control in hypertension is unknown. To investigate the role of SFO, we stereotaxically injected a recombinant adenovirus, encoding a Cre-recombinase and a GFP-reporter (AT1aR-KO-SFO), or GFP alone (AT1aR-WT-SFO), in the SFO of AT1aRflox mice. We validated the procedure of AT1aR deletion by visualizing GFP expression and by genomic PCR for AT1aR on SFO sections by laser capture microdissection. To investigate whether SFO is a mediator of AngII signalling, we injected fluorescent labelled AngII in the right femoral vein, observing by confocal microscopy analysis that it accumulates in the SFO. We measured BP by tail cuff, finding a significant reduction in mice with AT1aR KO in SFO after 28 days AngII infusion. We analyzed whether the immune*

activation is hampered in the spleen of these mice. By immunofluorescence analysis of T cell content in the splenic white pulp, AT1aR KO-SFO mice displayed reduced egression of T cells after chronic AngII, compared to WT mice. We found a significantly reduced infiltration of CD8 as an index of less renal damage. Our data demonstrate that Agtr1a receptors in the SFO are involved in the regulation of AngII-induced immune response, BP increase and renal damage as typical hallmarks of hypertension.

3. **Author: Jithin Kuriakose**; Co Authors: A.C. Montezano1, R. A. Lopes1, Angie YY Sin1, Delyth Graham1, G. S. Baillie1, R. M. Touyz1. Affiliations: Institute of Cardiovascular and Medical Sciences, University of Glasgow, UK**.** ***Title:*** *Molecular Mechanisms Involved in the Vascular Protective Effects of Mas1:ETBR Interaction. Mas1 and ETBR physically interact in endothelial cells inducing Ang-(1-7) vascular protection. High throughput screening of >20,000 druggable compounds identified several molecules that enhance Mas1:ETBR interaction, of these 2 were potent. These enhancers (Enh), named Enh3 and Enh4 were used to assess cellular and functional responses in vascular smooth muscle cells (VSMCs) from normotensive (WKY) and hypertensive (SHRSP) rats. Changes in vascular function were assessed by wire myography. VSMCs were exposed to Enh3 and Enh4 (10-5M) for short (5,15 and 30-min) time points. Expression of signalling molecules was assessed by immunoblotting and Ca2+ influx was evaluated using fluorescence microscopy. Mesenteric arteries were pre-incubated with Enh3 and 4 for 30 min prior to ET-1 induced contraction curve. In WKY VSMCs, Enh3 short term stimulation reduced basal ERK1/2 phosphorylation (26.8±5.9% vs veh, p<0.01), an effect absent in SHRSP VSMCs, while Enh4 had no effect. Short-term exposure to Enh4, but not Enh3, reduced basal MLC20 phosphorylation (54.9±7.5% vs veh, p<0.001) in WKY but not in SHRSP VSMCs. In SHRSP VSMCs, Enh3, but not Enh4, reduced basal AKT phosphorylation (63.5±8.9% vs veh, p<0.001). ET-1 induced Ca2+ influx in WKY and SHRSP VSMCs were unaffected by Enh3 and 4 preincubations. Enh3 and 4 reduced ET-1 induced contraction (Emax (Mn): Veh- 6.90±0.95; Enh3- 4.14±0.78: Enh4– 3.94±1.43) in female WKY rat vessels. Enh3 and 4 respectively attenuated mitogenic and pro-contractile signalling in WKY and SHRSP VSMCs, and does not increase Ca2+ signalling, important in VSMC contraction. These findings identify a potential new strategy in vasoprotection.*

4. **Author: Bakhta Fedlaoui;** Co Authors; G.E.M. Boari[1], O. Scarano[1,2], B. Mangili[1,2], A. Corbani[1,2], F. Leidi[1,2], B. Accordini[1,2], F. Napoli, C. Ghidelli[1,2], G. Archenti[1,2], D. Turini[1], M. Saottini[1], V. Guarinoni[1], G. Ferrari-Toninelli[1], F. Manzoni[1], A. Marengoni[2], D. Rizzoni[1,2]. Affiliations: *PARCC, INSERM, Université de Paris, Paris, FRANCE °Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, Va 20147, USA ^ PARCC, INSERM, Université de Paris and Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Service de Génétique, Paris, FRANCE ²equal contributio ***Title:*** *Development of an adrenocortical cell model of calcium balance modulation to decipher the molecular consequences of mutations responsible for primary aldosteronism. Primary aldosteronism (PA) is the most common and curable form of secondary arterial hypertension. Over the last few years, major advances have been made in PA with the identification of somatic and constitutional mutations in genes coding for ion channels and ATPases, making PA a channelopathy. These mutations converge towards a stimulation of calcium signalling, the main effector of the regulation of aldosterone biosynthesis. The objective of this work is to evaluate the molecular mechanisms underlying the development of PA by modulating calcium signalling using chemogenetic tools. We have developed an adrenocortical cell model expressing a chimeric receptor formed from the ligand-binding domain of the α7 acetylcholine receptor fused with the ionic pore of the serotonin receptor 5HT3α. The activation of this receptor by a synthetic molecule (PSEM-817) induces sodium entry into the cell. Stimulation of this channel by different concentrations of PSEM-817 (10-9 to 10-5 M) induces a significant increase in intracellular calcium content, greater than that induced by potassium (12 mM). This stimulation of calcium signalling does not induce changes in cell proliferation, but is responsible for an increase in CYP11B2 expression after 24h of treatment only for the highest doses of PSEM-817 (10-7 to 10-5 M). We have generated, for the first time, a cell model*

in which we can modulate intracellular calcium concentrations in an inducible manner. These cells are a valuable tool for a better understanding of the molecular consequences of altered ion balance and calcium signalling in the pathophysiology of PA.

5. **Author: Krithika Swaminathan**; Co Authors: Aycen Koc, Sabine Kaczmarek, Kristin Lehnert, Ines Urbaneck, Ulf Landmesser, Stephan B. Felix, Marcus Dörr, Martin Bahls, Nicolle Kränkel. Affiliations: Charité–Universitätsmedizin, Berlin;University Medicine Greifswald;DZHK- Partner Sites Berlin and Greifswald. ***Title:*** *Immune-metabolome response to a single exercise exertion reveals dysfunctional metabolic recovery in heart failure. Background: Increased systemic inflammation and metabolic dysfunction are observed in heart failure with reduced ejection fraction (HFrEF). On the other hand, cardiorespiratory exercise testing (CPET) exerts a physical challenge thereby initiating activation of immune system and several metabolic pathways. Aim: To characterize the inflammatory and metabolic alterations of HFrEF patients in response to an acute exercise challenge, and after 2 hours of rest. Methods: Participants with HFrEF (n = 16), age and sex matched controls (CON, n = 13) were investigated at baseline (T1), immediately after CPET (T2), and after 2 hours of resting (T3). Clinical and physiological parameters, leucocyte profile, plasma cytokines and metabolites were assessed along with inflammatory and metabolic parameters at all three time points. Results and Discussion: Cardiovascular risk profile as well as leukocyte, cytokine and metabolic parameters at T1 was similar in CON and HFrEF. Immediately after CPET, lactate, natural killer (NK) and NK T cell blood counts were significantly increased in both groups. In HFrEF specifically, platelet aggregates with NK cells, CD8+ cytotoxic T cells and "classical" CD14++CD16-monocytes, 58 different phosphatidylcholines and 21 triglycerides were increased. At T3 almost all altered parameters returned to baseline in CON. In HFrEF, blood counts and morphological markers of inflammatory effector cell types, including CD8+ T cells and neutrophils remained elevated. Conclusion: Although no differences in metabolic and inflammatory parameters between HFrEF and CON were evident at baseline, our data supports that the response to physical challenge might reveal early pathologic changes and aid metabolically active therapy development.*

6. **Author: Shunxin Jin[1];**Co Authors: Simone.J.P.M. Eussen[2,3], Casper G Schalkwijk[1], Coen DA Stehouwer[1], Marleen MJ van Greevenbroek[1]. Affiliations: [1] Dept. of Internal Medicine, CARIM School for Cardiovascular Diseases, [2] Dept. of Epidemiology, CARIM School for Cardiovascular Diseases, [3] CAPHRI School for Public Health and Prim Care, Maastricht University and Maastricht University Medical Centre, the Netherlands. ***Title:*** *The cross-sectional association of complement factor D with cardiovascular disease. Background: The complement system, particularly the alternative complement pathway, may contribute to vascular damage and development of cardiovascular disease (CVD). We investigated the association of Factor D, the rate-limiting protease in alternative pathway activation, with adverse cardiovascular outcomes. Methods: In 3541 participants (51.7% men, 59.9±8.3 years, 28.0% type 2 diabetes [T2D], oversampled) we measured low-grade inflammation (LGI, composite score, in SD), endothelial dysfunction (ED, composite score, in SD), carotid intima-media thickness (cIMT), ankle-brachial index (ABI), CVD and plasma concentrations of factor D. We conducted multiple linear and logistic regression to investigate the association of factor D with LGI, ED, cIMT and ABI and CVD, adjusting for potential confounders. Results: Adjusted for age, sex and T2D, the associations of factor D with LGI, ED, cIMT, ABI and CVD were 0.254 SD [95%CI 0.222;0.285], 0.218 SD [0.186;0.250], -4.45 ㎛ (-10.67;1.76), -0.008 [-0.011; -0.004] and OR 1.18 [1.08;1.29], respectively. After additional adjustment for smoking, education level and waist circumference, factor D remained significantly and positively associated with LGI (0.170 SD [0.139;0.201]), and CVD [1.11 (1.02;1.22)], independently and inversely with ABI (-0.004, [-0.007;0.000]) and non-significantly with cIMT (-5.07, [-11.52; 1.38]). The strength of the association between factor D and ED differed between participants with (0.108 SD [0.040;0.176]) and without T2D (0.203 SD [0.168;0.238], Pinteraction <0.005). Conclusion: Plasma factor D independently associated with LGI, ED, ABI, and CVD but not with cIMT. Hence, greater plasma*

factor D concentration in CVD may potentially induce complement activation which, in turn, might contribute to further disease progression.

7. **Author: María Galán;** Co Authors: Juan Antonio Arroyo, Attila Tobor, Laia Cañes, Miquel Navas-Madroñal, Elisabeth Pérez-Chust, José Martínez-González, Cristina Rodriguez, Júlia Caparrós. Affiliations: Institut de Investigació Biomèdica Sant Pau. ***Title:*** *Inhibition of endoplasmic reticulum stress as a therapeutic strategy to limit cardiac hypertrophic remodeling associated to hypertension. Background: Endoplasmic reticulum (ER) homeostasis is disturbed by the accumulation of misfolded proteins, eliciting a cellular response called ER stress. Persistent ER stress contributes to the pathophysiology of a myriad of cardiovascular diseases, including those linked with hypertension, such as cardiac hypertrophy. Aim: our objective was to characterize the ER stress response in human hypertensive hearts and to elucidate whether pharmacological and genetic approaches aiming to reduce cardiac ER stress limit hypertensive cardiac remodeling. Methods: Hypertension was induced in mice by angiotensin II (Ang II) infusion. Animals were infected with adeno-associated virus (AAV-9) targeting ATF4 or ATF6.Cardiac function and blood pressure (BP) were monitored and mRNA and protein levels assessed by Western-blot, qPCR and ELISA. Results: Classic ER stress inhibitors improved survival and reduced BP and heart hypertrophy in Ang-II-infused ApoE−/− mice. C57BL/6J males injected with AAV-9 expressing shRNAs against ATF4 or ATF6 showed reduced cardiac hypertrophy and BP in response to Ang II infusion. Additionally, CHOP deficiency in ApoE−/− mice prevented the hypertrophic effects of Ang II, reducing LV mass, HW/BW and BP. Interestingly, higher expression of ER stress markers was detected in hearts from hypertensive subjects compared with healthy donors, although no differences were found between subjects with and without myocardiopathy. Finally, we determined that circulating levels of GDF15, a CHOP-target gene, may be a good prognostic biomarker of cardiovascular events in hypertensive patients. Conclusions: our findings evidence that targeting ER stress with different therapeutic approaches reduces blood pressure and cardiac remodeling in response to hypertension.*

Oral Communication Session 1

8. **Author: Shunxin Jin[1];** Co Authors: Simone.J.P.M. Eussen[2,3], Casper G Schalkwijk[1], Coen DA Stehouwer[1], Marleen MJ van Greevenbroek[1]. Affiliations: [1] Dept. of Internal Medicine, CARIM School for Cardiovascular Diseases, [2] Dept. of Epidemiology, CARIM School for Cardiovascular Diseases, [3] CAPHRI School for Public Health and Prim Care, Maastricht University and Maastricht University Medical Centre, the Netherlands. ***Title:*** *The cross-sectional association of complement factor D with cardiovascular disease. Background: The complement system, particularly the alternative complement pathway, may contribute to vascular damage and development of cardiovascular disease (CVD). We investigated the association of Factor D, the rate-limiting protease in alternative pathway activation, with adverse cardiovascular outcomes. Methods: In 3541 participants (51.7% men, 59.9±8.3 years, 28.0% type 2 diabetes [T2D], oversampled) we measured low-grade inflammation (LGI, composite score, in SD), endothelial dysfunction (ED, composite score, in SD), carotid intima-media thickness (cIMT), ankle-brachial index (ABI), CVD and plasma concentrations of factor D. We conducted multiple linear and logistic regression to investigate the association of factor D with LGI, ED, cIMT and ABI and CVD, adjusting for potential confounders. Results: Adjusted for age, sex and T2D, the associations of factor D with LGI, ED, cIMT, ABI and CVD were 0.254 SD [95%CI 0.222;0.285], 0.218 SD [0.186;0.250], -4.45 ㎛ (-10.67;1.76), -0.008 [-0.011; -0.004] and OR 1.18 [1.08;1.29], respectively. After additional adjustment for smoking, education level and waist circumference, factor D remained significantly and positively associated with LGI (0.170 SD [0.139;0.201]), and CVD [1.11 (1.02;1.22)], independently and inversely with ABI (-0.004, [-0.007;0.000]) and non-significantly with cIMT (-5.07, [-11.52; 1.38]). The strength of the association between factor D and ED differed between participants with (0.108 SD [0.040;0.176]) and without T2D (0.203 SD [0.168;0.238], Pinteraction <0.005). Conclusion: Plasma factor D*

independently associated with LGI, ED, ABI, and CVD but not with cIMT. Hence, greater plasma factor D concentration in CVD may potentially induce complement activation which, in turn, might contribute to further disease progression.

9. **Author: Raquel Rodrigues Díez;** Co Authors: Constanza Ballesteros-Martinez[1,2], Rosa Moreno-Carriles[4], Mercedes Salaices[1,2,3], Ana M. Briones[1,2,3]. Affiliations: [1]Departamento de Farmacología, Facultad de Medicina, Universidad Autónoma de Madrid. [2]Instituto de Investigación Hospital Universitario La Paz (IdiPaz), Madrid, Spain. [3]CIBER de Enfermedades Cardiovasculares, Spain. [4]Servicio de Angiología y Cirugía vascular. Hospital Universitario La Princesa. Madrid, Spain. ***Title:*** *RvD2 prevents abdominal aortic aneurysm formation and ameliorates vascular function in Angiotensin II-treated obese mice. Inflammation is a key feature in abdominal aortic aneurysm (AAA). Resolution is the ideal outcome of inflammation and is performed by pro-resolving lipid mediators such as lipoxins, resolvins, protectins, and maresins. Resolution deficiency can lead to chronic inflammation. Recent studies demonstrate a protective role of some proresolvin mediators in experimental models of aneurysm. We aimed to study the effect of Resolvin D2 (RvD2) on aneurysm onset and development and the associated vascular alterations. For this, AAA was induced in C57BL6 male mice by 4 month of high fat diet (60%) and Angiotensin II (AngII) (1.4 mg/kg/day) infusion during the last month. One group of mice was treated with RvD2 (4µg/kg) every 48h, from the day before AngII infusion until the end of the model. Mice fed a standard diet were used as controls. Obese mice treated with AngII presented an incidence of AAA of more than 80%, elevated blood pressure, and cardiac hypertrophy that was reduced by RvD2. RvD2 treatment reduced abdominal aortic dilatation (determined by magnetic resonance imaging) and improved aortic remodeling. Moreover, RvD2 improved altered contractile responses and endothelial dysfunction induced by AngII in aorta and mesenteric resistance arteries and decreased the expression of inflammatory markers in aorta and perivascular adipose tissue. In aorta of patients, we observed a positive correlation between the presence of AAA and the expression of genes related to the synthesis and signaling of RvD2 and pro-inflammatory genes. These results suggest that RvD2 administration might be a therapeutic option in the management of aneurysm.*

10. **Author: Silke Griepke Dam Nielsen[1];** Co Authors: M. P. Fonseca [1], E. Grupe[1], L. B. Steffensen[1], J. S. Lindholt[2], J. Stubbe[1], D. F. J. Ketelhuth[1,3]. Affiliations: [1)] Department of Cardiovascular and Renal Research, Institute of Molecular Medicine, University of Southern Denmark, Denmark; [2)] Department of Cardiothoracic and Vascular Surgery, Odense University Hospital, Denmark; [3)] Division of Cardiovascular Medicine, Center for Molecular Medicine, Department of Medicine, Karolinska Institute, Karolinska University Hospital, Sweden. ***Title:*** *Inhibition of pyruvate dehydrogenase kinase prevents abdominal aortic aneurysm formation in mice. Background: Abdominal aortic aneurysm (AAA) is a life-threatening condition, where inflammation has been pinpointed as the major driver. Currently, AAA treatment relies exclusively on surgical interventions, with no proven pharmacological therapies to prevent growth or rupture. Immunomodulation by pharmacological reprogramming of immune cell metabolism has been demonstrated in the context of several inflammatory diseases. Whether the same strategy could be used in AAA remains allusive. Aim: We hypothesized that inhibition of pyruvate dehydrogenase kinase (PDK), a gatekeeper enzyme connecting glycolysis and the TCA cycle, in immune cells will promote anti-inflammatory responses preventing AAA formation. Methods: AAA was induced in male C57BL/6J mice by intraluminal infusion of porcine pancreatic elastase (PPE) in the infrarenal aorta. The PDK-inhibitor dichloroacetate (DCA; 1 mg/mL) was administered ad libitum through the drinking water starting one week before AAA induction, and through two consecutive weeks before sacrifice. In parallel, control mice received regular drinking water. Results: DCA treatment significantly reduced by 22.6% the outer maximum diameter and by 18.4% the luminal circumference of the infrarenal aorta of DCA-treated mice vs controls. Histology of AAA specimens revealed that DCA substantially preserved the elastic laminae structure in the aortas, and significantly increased medial alpha-smooth muscle actin content by 41.4%. Surprisingly,*

DCA did not affect macrophage infiltration. Conclusion: Inhibition of PDK with DCA is an effective therapy to limit AAA expansion induced by PPE. The current data suggest PDK inhibition as an effective modulator of smooth muscle cell responses that should be further studied.

11. **Author: Karla Bianca Neves;** Co Authors: R. Alves-Lopes, N.N. Lang, A.C. Montezano, R.M. Touyz. Affiliations: Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow – UK. ***Title:*** *PARP/TRPM2 inhibition attenuates VEGF inhibitor-induced vascular dysfunction. VEGF inhibitors (VEGFi) are used as anti-angiogenic drugs in cancer treatment, which commonly cause hypertension. Clinical findings suggest that the combination of VEGFi with another anti-cancer drug, olaparib (PARP inhibitor [PARPi]), may attenuate the development of hypertension, although mechanisms are still unknown. PARP is key for the activation of TRPM2, a redox-sensitive Ca2+ channel, which is associated with hypertension-induced vascular dysfunction. We hypothesized that PARPi attenuates VEGFi-induced vascular injury through TRPM2/Ca2+-dependent pathways. Human vascular smooth muscle cells (hVSMC), human aortic endothelial cells (HAEC), and mouse mesenteric arteries/aorta were exposed to axitinib (VEGFi) alone or in combination with olaparib. Axitinib increased ET-1-induced Ca2+ influx (AUC: 17541±4708 [Ct] vs. 22249±1438 [Axi]) in hVSMC. These effects were blocked by olaparib and 8-Br-cADPR. Axitinib induced phosphorylation of MLC20 in hVSMC (a.u.: 0.028±0.02 [Ct] vs. 0.04±0.01 [Axi]) and aorta (a.u.: 0.3±0.01 [Ct] vs. 0.5±0.001 [Axi]). U46619- and ET-1-induced vasoconstriction were increased by axitinib (% KCl-U4: 101.2 [Ct] vs. 141.4 [Axi]; ET-1: 122.6 [Ct] vs. 152.5 [Axi]), an effect not observed with axitinib plus olaparib. Axitinib reduced ACh-induced vasodilation (% relaxation: 70.5 [Ct] vs. 34.8 [Axi]) and eNOS activity (Thr495-a.u.: 0.99±0.35 [Ct] vs. 1.35±0.10 [Axi]). These effects were blocked by olaparib. VEGF-induced NO production is blocked by Axitinib, which is not observed with axitinib plus Olaparib. Our data indicate that PARP/TRPM2 inhibition attenuates axitinib-mediated vascular dysfunction and normalizes VEGFi-induced hVSMC/HAEC signalling dysfunction. We define a putative vasoprotective effect of olaparib that may attenuate vascular injury and hypertension induced by VEGFi in cancer treatment.*

12. **Author: Eline Berends;** Co Authors: Naima Amiri [1,2], Marjo PH van de Waarenburg [1,2], Jean LJM Scheijen [1,2], Denise JHP Hermes [3,6], Robert J van Oostenbrugge [2,4], Sébastien Foulquier [2,5,6]*, Casper G Schalkwijk [1,2] *. Affiliations: [1]Department of Internal Medicine, Maastricht University, Netherlands. [2]School for Cardiovascular Diseases (CARIM), Maastricht, Netherlands. [3]Department of Psychiatry and Neuropsychology, Maastricht University, Netherlands. [4]Department of Neurology, Maastricht University medical Centre, Netherlands. [5]Department of Pharmacology and Toxicology, Maastricht University, Netherlands. [6]School for Mental Health and Neuroscience, Maastricht, Netherlands. *Shared last-authorship. ***Title:*** *Supplementation of methylglyoxal in drinking water does not affect the cerebral microvasculature and cognitive function in healthy young mice. Background: Diabetes is associated with cerebral small vessel disease (cSVD) and cognitive impairment, however, the underlying mechanism remains unclear. Methylglyoxal (MGO), a by-product of glycolysis and a precursor of advanced glycation end products (AGEs), is increased in diabetes and associated with microvascular dysfunction. Previously, our group showed that MGO is increased in brain tissue of diabetic rats. Aim: To investigate the impact of increased MGO levels on brain microvascular inflammation and cognitive function. Methods: 2-3 months old male C57Bl/6J mice were treated with MGO (50mmol/l, drinking water) or not (control) for 3 months (n=17/group), and underwent series of cognitive tests. Plasma and brain MGO and AGEs were measured by UHPLC-MS/MS, plasma inflammatory markers by ELISA and cortical microvessels were isolated for mRNA expression. Results: In the MGO group, plasma MGO was increased 2-fold ($p<0.0001$) and free plasma MGO-derived hydroimidazolone-1 (MG-H1) and Nε-(1-carboxyethyl)lysine (CEL) were increased 1.2-fold ($p=0.01$) and 1.7-fold ($p=0.01$), respectively. In brain tissue, there was a 1.4-fold and 1.1-fold increase in free MG-H1 ($p=0.02$) and CEL ($p=0.001$) compared to control. Cognitive function remained unchanged after MGO treatment. MGO did not change expression of plasma inflammatory markers*

IFN-γ, IL-10, IL-1β, IL-6, TNF-α and CXCL1. In cortical microvessels, expression of inflammatory genes VCAM-1, ICAM-1, sirtuin-1 and AGE receptor, were unchanged. Discussion/Conclusion: Plasma MGO, plasma and brain MGO-derived AGEs were increased by MGO intake to a level comparable to diabetic patients with no impact on microvascular inflammation and cognitive function. Circulating MGO by itself does not cause microvascular inflammation nor cognitive decline.

13. **Author: Sara Jiménez González;** Co Authors: S. Jiménez-González[1], F.V. Souza-Neto[1], B. Delgado-Valero[1], R. Jurado-López[1], M. Genty[1], A. Romero-Miranda[1], C. Rodríguez[2,3,4], M.L. Nieto [4,5], E. Martínez-Martínez [1,4], V. Cachofeiro[1,4]. Affiliations: [1]Departamento de Fisiología, Facultad de Medicina, Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM), Universidad Complutense de Madrid, 28040 Madrid, Spain. [2]Institut de Recerca del Hospital de la Santa Creu i Sant Pau, 08025 Barcelona, Spain. [3]Instituto de Investigación Biomédica Sant Pau (IB Sant Pau), 08025 Barcelona, Spain. [(4)] Ciber de Enfermedades Cardiovasculares (CIBERCV), Instituto de Salud Carlos III, 28220 Majadahonda, Spain. [(5)] Instituto de Biología y Genética Molecular, CSIC-Universidad de Valladolid, 47002 Valladolid, Spain. ***Title:*** *The mitochondrial oxidative stress-endoplasmic reticulum stress axis in the development of cardiovascular fibrosis in obese rats. Background: Obesity is associated with cardiovascular fibrosis, which plays an important role in the development of diastolic dysfunction and arterial stiffness. Oxidative stress and endoplasmic reticulum (ER) stress have been suggested as possible mechanisms involved, although their interaction is not well established. Aim(s)/Objective(s): Evaluate the role of mitochondrial oxidative stress and its association with ER stress activation in the progression of obesity-related cardiovascular fibrosis. Methods: Male Wistar rats were fed with standard diet (CT; 3.5% fat) or high fat diet (HFD; 35% fat) for 7 weeks, and treated with the mitochondrial antioxidant MitoQ (200 μM). Cardiac fibroblasts and vascular smooth muscle cells (VSMCs) were treated with Angiotensin II (Ang II; 10-6 M) in presence or absence of MitoQ (5 nM) or the ER stress inhibitor, 4-phenyl butyric acid (4-PBA; 4 μM). Results: Obese animals presented cardiovascular fibrosis accompanied by increased levels of ECM component collagen I and profibrotic mediators connective tissue growth factor and transforming growth factor-β. These changes were associated with ER stress activation, characterized by increased levels of immunoglobulin binding protein (BiP), protein disulfide-isomerase A6 (PDIA6) and CCAAT-enhacer-binding homologous protein (CHOP). MitoQ was able to prevent these alterations at cardiac and aortic levels. At cellular level, the profibrotic and prooxidants actions of Ang II were prevented by both MitoQ and 4-PBA. Conclusions: The data shows a crosstalk between mitochondrial oxidative stress and ER stress activation, which mediates the development of cardiovascular fibrosis in the context of obesity and in which Ang II can play a relevant role.*

14. **Author: Augusto C Montezano;** Co Authors: Livia De Lucca Camargo, Sheon Samji, Karla Neves, Francisco Rios, Rheure A Lopes, Wendy Beattie, Imogen Herbert, Vanessa Herder, Agnieszka M Szemiel, Steven McFarlane, Massimo Palmarini, David Bhella, Rhian M Touyz. Affiliations: Institute of Cardiovascular and Medical Sciences and MRC - University of Glasgow Centre for Virus Research, University of Glasgow, UK. ***Title:*** *ACE2 is involved in SARS-CoV-2 induced endothelial cell inflammation independent of viral replication. Background: COVID-19 association with cardiovascular disease is thought to be due to endothelial cell inflammation. ACE2 interactions with SARS-CoV-2 spike protein S1 subunit is important to viral infection. Aims: Here we questioned whether SARS-CoV-2 induces vascular inflammation via ACE2 and whether this is related to viral infection. Methods: Human microvascular endothelial cells (EC) were exposed to recombinant S1p (rS1p) 0.66 μg/mL for 10 min, 5h and 24h. Gene expression was assessed by RT-PCR and levels of IL6 and MCP1, as well as ACE2 activity, were assessed by ELISA. Expression of ICAM1 and PAI1 was assessed by immunoblotting. ACE2 activity was blocked by MLN4760 (ACE2 inhibitor) and siRNA. Viral infection was assessed by exposing Vero E6 (kidney epithelial cells; pos ctl) and EC to 105 pfu of SARS-CoV-2 where virus titre was measured by plaque assay. Results: rS1p increased IL6 mRNA (14.2±2.1 vs. C:0.61±0.03 2^-ddCT) and levels (1221.2±18.3 vs. C:22.77±3.2 pg/mL); MCP1*

mRNA (5.55±0.62 vs. C:0.65±0.04 2^-ddCT) and levels (1110±13.33 vs. C:876.9±33.4 pg/mL); ICAM1 (17.7±3.1 vs. C:3.9±0.4 AU) and PAI1 (5.6±0.7 vs. C: 2.9±0.2), p<0.05. MLN4760, but not rS1p, decreased ACE2 activity (367.4±18 vs. C: 1011±268 RFU, p<0.05) and blocked rS1p effects on ICAM1 and PAI1. ACE2 siRNA blocked rS1p-induced IL6 release, ICAM1, and PAI1 responses as well as rS1p-induced NFκB activation. EC were not susceptible to SARS-CoV-2 infection, while the virus replicated well in Vero E6. Conclusion: In conclusion, rS1p induces an inflammatory response through ACE2 in endothelial cells; an effect that was independent of viral infection.

Oral Communication Session 2

15. **Author: Rhéure Alves-Lopes;** Co Authors: Karla B Neves, Augusto C Montezano, Sheon Samji, Christian Delles, Rhian M Touyz. Affiliations: University of Glasgow, Institute of Cardiovascular and Medical Sciences (ICAMS) - UK. ***Title:*** *Redox activation of PARP/TRPM2 signalling contributes to vascular damage induced by high salt diet without influencing blood pressure. By mechanisms not fully elucidated, high salt diet (HSD) has deleterious effects on the vasculature. We demonstrated coupling between Na+/Ca2+ in VSMCs, where PARP-regulated TRPM2 (redox-sensitive Ca2+ channel), plays a role. Increased [Ca2+]i also contributes to inflammasome assembly, which dysregulates vascular function. We hypothesized that HSD induces a pro-oxidant environment, contributes to PARP-induced TRPM2-activation, Ca2+ influx and inflammasome assembly, leading to vascular damage. WKY rats were treated with 1%HSD (3w). Blood pressure was assessed by tail-cuff, vascular reactivity was assessed in mesenteric arteries and calcium influx, ROS generation, inflammasome and pro-contractile marker in vascular smooth muscle cells (VSMCs) in presence and absence of HS medium (HSM-NaCl140mM). HSD did not increase blood pressure (BP), but vascular contractility was exaggerated (Emax(mN): WT 10.2±0.7vs15.2±1.7 HSD), effect reversed by PARP and TRPM2 inhibitors. In VSMCs, HSM behaves as a pro-oxidant agent (ROS-AU: Control 77.5±2.8vs130±13.9 HSM), leading to increased [Ca2+]i (AUC:Control 25562.4±880.5vs30924.8±1263.8 HSM) and activation of myosin light chain (pMLC: Control 99.3±1.01vs626.9±71.28 HSM), by mechanisms dependent on ROS and PARP/TRPM2 activation. HSM increased expression of inflammasome components NLRP3 (2ΔΔCt:Control 1.2±0.01vs1.85±0.2 HSM), ASC (2ΔΔCt:Control 1.0±0.01vs1.4±0.1 HSM) and Caspase 1 (2ΔΔCt:Control 1.1±0.03 vs 2.4±0.5 HSM), which was prevented by ROS scavenger Tiron and PARP inhibitor. In conclusion, HSD-induced vascular hypercontractility involves ROS activation of PARP/TRPM2 signalling. Activation of PARP/TRPM2 was associated with increased [Ca2+]I, activation of pro-contractile signaling and inflammasome assembly. Normal BP in HSD-fed rats in the presence of vascular damage suggests that ROS/PARP/TRPM2 signaling induced by salt influences vascular function independently of BP elevation.*

16. **Author: Tianyu Hang;** Co Authors: Corrales S, Azkargorta M, Elortza F, Martínez-Chantar M, Egido J and Lorenzo O. Affiliations: IIS-Fundación Jiménez Díaz, UAM. ***Title:*** *mTORC1 activation and mitochondrial dysfunction in diabetic and hypertensive cardiomyopathy. Background: Type 2 Diabetes(T2DM) and hypertension (HTN) are major cardiomyopathy causes. Patients with both pathologies may have even a worse prognosis than those with any alone. Aim: Characterized the cardiac proteins' alteration in diabetic and hypertensive cardiomyopathy. Study the potential mechanisms involved in cardiac mitochondrial dysfunction. Methodology: Cardiac biopsies were isolated from patients with T2DM and /or HTN. Differential protein expression was evaluated by proteomics (hybrid trapped ion mobility spectrometry) and PEAKS software. Implication of molecular pathways was predicted by Ingenuity Pathway Analysis (IPA, Qiagen). Cultured cardiomyocytes were used to reveal relevant pathways alteration under different conditions. Results: Cardiac samples from HTN and T2DM patients shows more proteins altered than that in HTN (increase:117 Vs 4, decrease 549 Vs 41). Both groups induced the alteration mostly in metabolism (19%) and mitochondrial factors (42%), also pro-apoptosis and fibrosis. In cultured cardiomyocytes stimulated by excess of glucose*

(HG), fatty-acid (HF) and/or angiotensin-II we confirmed the expression of factors like Annexin A5, Decorin and NDUFA2. Simultaneously, HF with or without angiotensin-II, but not HG, enhanced P-P70S6(mTORC1 downstream mediator) while P-Akt (mTORC2 downstream mediator) was ameliorated by HF, but elevated by HG. Conclusion: The alteration of cardiac proteins under T2DM and HTN could be higher than after any pathology alone. At least the mitochondrial factors alteration may be responsible for cardiac dysfunction. mTORC1 complex activation may precede mitochondrial metabolic enzymes' reduction leading to ATP synthesis lacking and cardiac dysfunction. The cardiac mTOR-mitochondria axis regulation could be essential for heart-failure prognosis after T2DM and HTN.

17. **Author: Koen van der Laan;** Co Authors: K. van der Laan[1,2], M. van der Bruggen [1,2], A. Juttner [3], P. Spronck[4,] C. schalkwijk[5], A. Roks[3], T. Delhaas[1,2], B. Spronck[1,2,6], K. Reesink[1,2]. Affiliations: [1]. CARIM School for Cardiovascular Diseases, Maastricht University, Maastricht, The Netherlands; [2]. Department of Biomedical Engineering, Maastricht University, Maastricht, The Netherlands; [3].Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands; [4].Department of Internal Medicine, Maastricht University, Maastricht, The Netherlands; [5]. Design and Development, Innovatech-Europe BV, Maastricht, The Netherlands; [6].Department of Biomedical Engineering, School of Engineering & Applied Science, Yale University, New Haven, CT, USA. ***Title:*** *Pulsatile biaxial mechanical behavior at different levels of smooth muscle tone: A pilot study. Background Pulse wave velocity (PWV), a clinical measure of arterial wall stiffening, is associated with an increased risk of cardiovascular disease (1). Since PWV does not provide insight into the arterial wall's mechanical properties, dynamic biaxial mechanical ex vivo experimental studies are used to investigate intact vessel biomechanics vessels under in vivo-like conditions in more detail (2). Objective How vascular smooth muscle cell (VSMC) contraction influences dynamic vessel biomechanics remains poorly understood. Hence, we developed a dynamic biaxial mechanical testing setup in which VSMC tone can be modulated. Methods An excised left murine common carotid artery was mounted in the setup, submerged in HEPES saline buffer and brought to in vivo length. VSMC relaxation or contraction was induced by switching the buffer's potassium chloride concentration between 4.7 and 62.5 mM, respectively. After the vessel's diameter stabilized, pressure-diameter loops were recorded during dynamic pressurization from 80 to 100 (low-pulse pressure (PP)) and from 70 to 110 mmHg (high-PP) at 10-Hz. PWVs were derived from the pressure-diameter loops using the Bramwell-Hill relationship Results After contraction and relaxation, PWVs were 6.72 m/s (low-PP) and 4.10 m/s (high-PP), and 5.00 m/s (low-PP) and 4.24 m/s (high-PP), respectively. Diameters at minimum pressure were 18.5% (low-PP) and 11.3% (high-PP) lower after contraction than relaxation. Discussion Decreased diameters after contraction demonstrate a stiffer vessel, while high-PP PWV values suggest the opposite, demonstrating how interpreting 'PWV' as 'stiffness' may be misleading. Conclusion Our novel setup is suitable for investigating dynamic vessel biomechanics during induced VSMC contraction.*

18. **Author: Francisco J. Rios;** Co Authors: Augusto C Montezano, Livia L Camargo, Rheure A Lopes, Elihu Aranday-Cortes, John McLauchlan, Rhian M Touyz. Affiliations: MRC-Centre for Virus Research, University of Glasgow BHF-ICAMS, University of Glasgow. ***Title:*** *Spike protein 1 of SARS-CoV-2 induces inflammatory response in endothelial cells by increasing interferon stimulated responses. Introduction: Interferon-alpha (IFNα) and lambda3 (IFNL3) are produced during SARS-CoV-2 infection. They are the first line of immunity by increasing interferon-stimulated genes (ISGs). IFNs influence the expression of angiotensin-converting enzyme-2 (ACE2), the receptor for S-protein (SP1) of SARS-CoV-2. Here we hypothesized that in human microvascular endothelial cells (EC), IFNL3 and IFNα influence immune/inflammatory responses mediated by SP1. Methods: EC were stimulated with SP1(1µg/10^6 cells), IFNα(100ng/mL) or IFNL3(100IU/mL). Because ACE2, ADAM17 and TMPRSS2 are important for SARS-CoV-2 infection, cells were treated with inhibitors of ADAM17 (marimastat, 3.8nM), ACE2 (MLN4760, 440pM), and TMPRSS2 (camostat, 50µM). ISGs*

(ISG15, IFIT1, and MX1) was investigated by real-time PCR and protein expression by immunoblotting. Results: S1P increased expression of ISG15 (2-fold), IFIT1 (6-fold), MX1 (6-fold) (n=12, p<0.05). EC exhibited higher responses to IFNα (ISG15: 16-fold, IFIT1: 21-fold, MX1: 31-fold) than to IFNL3 (1.8-fold for ISG15, IFIT1, and MX1) (p<0.05). S1P increased mRNA for IL-6 (1.3-fold), TNFα (6.2-fold) and IL-1β (3.3-fold), effects that were maximized 100% by IFNα. Marimastat and TAPI-1 inhibited SP1 effects. IL-6 was increased by IFNα (1230 pg/mL) and IFNL3 (1124 pg/mL) vs control (591pg/mL). This was associated with increased p-Stat1 (134%), p-Stat2 (102%), p-ERK1/2 (42%). Nitric oxide production and phospho-eNOS (Ser1177) were reduced by IFNα and (40%) and IFNL3 (40%). Conclusions: SP1, IFNα and IFNL3 increased ISGs and IL-6 production in EC, processes that involve ADAM17. Inflammation induced by SP1 was amplified by IFNα. Our findings demonstrate that SP1 induces an endothelial immune/inflammatory response that may be important in endotheliitis associated with COVID-19.

19. **Author: Alaa B Abdellatif[1]**; Co Authors: Isabelle Giscos-Douriez[1] Fabio Fernandes-Rosa[1], Yunling Xu[1], Sheerazed Boulkroun[1]*, Maria-Christina Zennaro[1]. Affiliations: [1]Université de Paris, PARCC, INSERM, Paris, France [2]Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Service de Génétique, Paris, France *equal contribution. ***Title:*** *Vascular and hormonal interactions in primary aldosteronism. Primary aldosteronism (PA) is the most frequent form of secondary hypertension. We hypothesize that adrenal vascular changes may modify adrenal cortex structure and function leading to increased nodulation, creating a propitious environment for the occurrence of somatic mutations and development of aldosterone producing adenoma (APA). To understand the interaction between the vasculature and cell proliferation in the adrenal cortex, a new mouse model was generated, expressing the Cre recombinase specifically in the aldosterone-producing adrenal zona glomerulosa (ZG). Mice adrenal structure and vascularization were characterized under basal conditions and following high/low salt diets as well as dexamethasone treatment to challenge adrenocortical remodeling and function. In Cyp11b2-Cre::mTmG mice, expression of the Cre recombinase was restricted to the zona glomerulosa (ZG), its expression being absent in other tissues. While at day 1, only a few GFP+ cells were present in the ZG, at day 14 all cells of the ZG were GFP+. Subsequently, GFP+ cells transdifferentiate and colonize the zona fasciculata (ZF); this migration is faster in females than in males. Under high salt diet, Cyp11b2 expression was reduced, whereas under low salt diet, the ZG was expanded and Cyp11b2 expression increased. After 2 weeks of dexamethasone treatment, complete disorganization of the ZF was observed; podocalyxin staining showed concomitant disorganization of centripetal capillaries between columns of fasciculata cells. Three weeks after end of treatment, ZF and vessel regeneration was complete in both female and male mice. Experiments are currently ongoing to investigate how modifications of adrenal vascularization may affect adrenal structure and function.*

20. **Author: Licia Shamoon;** Co Authors: J. Espitia-Corredor[1,2], P. Dongil[1,3], I. Valencia[1,3], M. Menendez-Ribes[1], G. Díaz-Araya[2], C. Sánchez-Ferrer[1,3], C. Peiró [1,3]. Affiliations: [1].Department of Pharmacology and Therapeutics, Faculty of Medicine, Universidad Autónoma de Madrid, Madrid, Spain [2].Department of Pharmaceutical and Toxicological Chemistry, Faculty of Chemical and Pharmaceutical Sciences, University of Chile. [3].Instituto de Investigación Sanitaria del Hospital Universitario La Paz (IdiPAZ), Madrid, Spain. ***Title****: Resolvin E1 mitigates endothelial senescence induced by doxorubicin via modulating NLRP3 inflammasome activationBackground Vascular aging is associated with endothelial cell senescence, favoring low-grade inflammation, endothelial dysfunction, and cardiovascular diseases. Endothelial senescence arises from wide variety of endogenous and exogenous stressors including some anticancer agents such as doxorubicin. Recently, doxorubicin was linked to the innate immunity component NLRP3 inflammasome which is implicated in many vascular inflammatory disorders. There is a need for therapeutic tools to help cancer patients who have been exposed to cardiovascular toxic chemotherapy averting premature vascular complications. Objective We investigated whether resolvin E1 (RvE1), an endogenous lipid mediator*

of the inflammation resolution phase, could prevent doxorubicin-induced senescence in cultured human umbilical veins endothelial cells (HUVEC) with focus on a potential involvement of the NLRP3 inflammasome. Methods Cell senescence was quantified by senescence-associated-β-galactosidase (SA-β-gal) staining. The expression of senescence markers (γH2AX, p21, p53) and inflammatory markers (pP65, NLRP3) was determined via Western blot. NLRP3 inflammasome activation was determined by visualizing the formation of ASC specks by indirect immunofluorescence. Results Doxorubicin (25nmol/L) augmented the number of SA-β-gal positive HUVEC and the levels of γH2AX, p21 and p53 which were all reduced by RvE1 (10nmol/L). In doxorubicin-treated cells, RvE1 further reduced the expression of pP65 and NLRP3 proteins and the formation of ASC specks as did the inflammasome assembly inhibitor MCC950 (1µmol/L). Additionally, both MCC950 and interleukin-1 receptor inhibitor anakinra diminished SA-β-gal positive staining induced by doxorubicin. Conclusion RvE1 offers a novel therapeutic approach against doxorubicin-induced cardiovascular toxicity and subsequent age-related vascular disorders by counteracting endothelial senescence through the modulation of NLRP3-inflammasome activation.

21. **Author: S. Foulquier;** Co Authors: D. Kerkhofs, R. Helgers, H. van Essen, P. Leenders, D. Hermes, He Steinbusch, J. Prickaerts, E.A. Biessen, R.J. van Oostenbrugge, S. Foulquier. Affiliations: Pharmacology-Toxicology Dept, CARIM, MHeNS, Maastricht University. ***Title:*** *Amlodipine prevents microglia activation and cognitive dysfunction in aged hypertensive mice. Background Systolic blood pressure (SBP) and BP variability (BPv) are independent risk factors for cerebral small vessel disease (cSVD). Calcium-channel blockers (CCBs) may offer greater benefit against stroke and dementia than other anti-hypertensive classes possibly due to their superiority to reduce BPv. Beyond their BP effects, the impact of CCBs on hypertension-induced neuroinflammation remains unknown. While CCBs are able to reduce microglial activation in vitro, this has not been studied in vivo. Aim To study the ability of the CCB amlodipine to alleviate microglial activation and prevent cognitive dysfunction in aged hypertensive mice. Methods Hypertensive BPH/2J and normotensive BPN/3J mice were studied until 12 months of age. Hypertensive mice were untreated or received amlodipine (10 mg/kg). SBP and BPv were measured by telemetry and tail cuff plethysmography. Mice underwent repeated series of cognitive tasks. Brain IHC was performed to study BBB dysfunction, vascular density, hypoxia and microglial activation (CD68+Iba1+ cells; morphological analysis). Results and Discussion Amlodipine normalized SBP over the entire life span and decreased BPv. BPH/2J mice exhibited an impairment of short-term memory that was prevented by amlodipine. cSVD was characterized in BPH/2J by the presence of BBB leaks with microgliosis with no change in vascular density nor hypoxia. Microglial activation in BPH/2J was characterized by an increased number of CD68+ Iba1+ cells, increased soma size and shortened processes. This was prevented by amlodipine. Conclusion Amlodipine decreased microglial activation and prevented the impairment of short-term memory in aged hypertensive mice.*

Poster Session 1

22. **Author: Ilaria Caputo;** Co Authors: B. Caroccia, T. M. Seccia, G.P. Rossi. Affiliations: Specialized Center for Blood Pressure Disorders-Regione Veneto and Internal Emergency Medicine Unit, Department of Medicine-DIMED, University of Padua, Padua, Italy. ***Title****: ACE-2 expression is modulated by AT1R signaling in human bronchial epithelial cells. Background Angiotensin converting enzyme 2 (ACE-2) is the cellular receptor of SARS-CoV-2 that caused the COVID-19 pandemic. ACE inhibitors (ACEis) and angiotensin type 1 receptor (AT1R) antagonists (ARBs) were contended to raise ACE-2 expression, thus facilitating the SARS-CoV-2 infection and representing a potential risk factor in patients with cardiovascular comorbidities, but evidences supporting this hypothesis lack. Aim We investigated the effect of Angiotensin (Ang) II, ACE inhibitors (ACEIs) and Ang type 1 receptor (AT1R) antagonists (ARBs) on ACE-2 mRNA and protein levels in human*

epithelial bronchial cells. Methods Calu-3 cells, bronchial epithelial cells, were treated with 10μM irbesartan (ARB), 10 μM ramipril (ACEi) or 10 μM MLN-4760 (ACE-2 inhibitor) alone or on top of 100nM AngII. ACE-2 mRNA expression was evaluated by Digital droplet (dd)PCR after 12 hours treatment, ACE-2 protein levels were evaluated by immunoblotting after 48 hours treatment. Results We showed that AngII increased the mRNA (p=0.0007) and protein (p= 0.03) levels of ACE-2, while the ACEI ramipril and the ARB irbesartan did not. The effect of AngII on ACE-2 mRNA was abolished by irbesartan (p=0.007) and unaffected by ramipril. Blockade of ACE-2 enzymatic activity with MLN-4760 did not affect the AngII-induced ACE-2 increase. Conclusions As in human epithelial bronchial cells AngII upregulates ACE-2 expression via AT1R signalling and blockade of AngII formation and/or action can lower the SARS-CoV-2 receptor, and thus the spread of the COVID-19, these data support the beneficial effect of ACEIs and ARBs in patients with an activated renin-angiotensin-aldosterone system.

23. **Author: Maria Bloksgaard;** Co Authors: K. Rosenstand[1],*, I. Nissen[1],*, P. S. Jensen[2], S. Munthe[3], T. H. Nielsen[3.] Affiliations: [1] Cardiovascular and Renal Research Unit, Department of Molecular Medicine, University of Southern Denmark,[2] Odense Artery Biobank, Center for Individualized Medicine in Arterial Diseases (CIMA), Odense University Hospital, [3] Department of Neurosurgery, Odense University Hospital *These authors contributed equally. ***Title:*** *3D histology of cerebral aneurysms reveals spatial reorganization of the smooth muscle cells in the aneurysmal wall: Understanding the pathological and structural changes in aneurysmal tissues may inform preventive treatments. Here, we aimed to characterize excised cerebral aneurysm tissues using standard histological techniques and 3D multiphoton imaging with special attention to cellular organization. Cerebral aneurysmal tissue was excised from patients during open microsurgical clip occlusion of the aneurysm. Specimens were included after patients' (planned interventions) or relatives' (acute interventions) written informed consent. Specimens were collected in sterile, cold saline, fixed in formaldehyde, and stained with Alexa546-phalloidin (smooth muscle cytoskeleton), Alexa633-hydrazide (elastic fibers), 4',6-diamidino-2-phenylindole (DAPI, nuclei). 3D imaging was conducted using an Olympus FluoView1000 multiphoton microscope with XLPlan 25× water dipping objective, numerical aperture 1.05. The following wavelengths were used: DAPI λex 760nm / λem 420-500nm, collagen second harmonic generation (SHG): λex 840nm / λem 420–500 nm, Alexa546-phalloidin λex 840nm / λem, 560-600nm, Alexa633-hydrazide λex 840nm / λem 660-690nm. Following 3D imaging, specimens were embedded in paraffin, sectioned, and stained with Massons' trichrome stain. 3D imaging of specimens was superior in providing information on the organization of the aneurysmal wall. Specimens from ruptured aneurysms had a hardly recognizable arterial wall structure, while specimens from non-ruptured aneurysms revealed a smooth muscle layer organized in a honeycomb-like network. Histological sections confirmed that the wall of non-ruptured aneurysms was more organized but did not reveal the bidirectionality of the vascular smooth muscle. We propose using 3D imaging for future studies on the driving forces of aneurysm wall remodeling to gain information on the spatial reorganization of the smooth muscle cells.*

24. **Author: Igor Maciel Souza-Silva*;** Co Authors: Kenneth Kjærgaard*, Christina Mortensen#, Tore Bjerregaard Stage#, Ulrike Muscha Steckelings*. Affiliations: *Department of Cardiovascular and Renal Research, Institute of Molecular Medicine, University of Southern Denmark, Odense C, Denmark. #Clinical Pharmacology and Pharmacy, Department of Public Health, University of Southern Denmark, Odense C, Denmark. ***Title:*** *A high-throughput nitric oxide measurement assay to evaluate AT2 receptor activation in vitro reveals that angiotensin-(1-5) is an AT2 receptor agonist. Background: The angiotensin AT2-receptor (AT2R) is a key component within the protective arm of the renin-angiotensin system (RAS), being involved in nitric oxide (NO) production and vasodilation. No high-throughput assay is available to identify AT2R agonists in vitro, which could explain the low number of AT2R selective ligands in drug development programs. Objective: To design and validate a high-throughput method for detection of AT2R activation in vitro. Methods: NO release was selected*

as readout for AT2R activation in AT2R transfected (AT2R-CHO) and non-transfected (NT-CHO) CHO cells using DAF-FM (5x10-6 mol.L-1) as NO probe. Cells were seeded on 24-well plates (20000 cells/well) and stimulated for 15 minutes with C21 or Ang II (AT2R agonists, 10-6 mol.L-1), Ang-(1-5) (unknown biological status, 10-6 mol.L-1) or Ang-(1-7) (Mas-receptor agonist, 10-7 mol.L-1). After fixation of cells, fluorescence signals were captured by fluorescence microscopy using an automated imaging system (ImageXpress Pico, Molecular Devices, San Jose, USA) and image analysis by ImageJ. Results: In CHO-AT2, C21 (□34.78□12.09%), Ang II (□28.76□17.65%) and Ang-(1-5) (□78.00□23.82%) increased NO release (unpaired t-test, p≤0.05 vs control, ≥ 3 independent experiments), while Ang-(1-7) had no effect. In NT-CHO, none of the compounds stimulated NO release, indicating that the responses in CHO-AT2 were AT2R specific. Conclusion: Measurement of NO release from AT2R-CHO by DAF-FM fluorescence in an automated way is suitable as high-throughput assay for the identification of AT2R-agonistic compounds in vitro. Application of the assay revealed that Ang-(1-5), which is regarded as an inactive metabolite of Ang II, has AT2R agonistic properties.

25. **Author: Jairo Lumpuy-Castillo[1];** Co Authors: Claudia Vales-Villamarín Fernández[2], Iris Pérez Nadador[2], Leandro Soriano Guillén[3], Óscar Lorenzo González[1], Carmen Garcés Segura[2]. Affiliations: [1].Laboratory of Diabetes and Vascular pathology, IIS-Fundación Jiménez Díaz, UAM, Madrid, Spain [2].Lipid Research Laboratory, IIS-Fundación Jiménez Díaz, Madrid, Spain [3].Department of Pediatrics, IIS-Fundación Jiménez Díaz, Madrid, Spain. ***Title:*** *Association of ACE2 polymorphisms with obesity and elevated lipids in female adolescents. Background: The role of the angiotensin-converting enzyme 2 (ACE2) as mitochondrial protector and angiotensin-(1-7) producer has been previously described. However, some ACE2 gene polymorphisms may associate with the development of diabetes, dyslipidemia, and essential hypertension. Aims: We examined the potential relationship between some ACE2-SNPs and cardiovascular risk factors in Spanish adolescents. Methods: A cross-sectional observational study was conducted in a cohort of 461 girls and 412 boys (12-16 years old). By RT-PCR, we examined the presence of five SNPs of the ACE2 gene (rs4646188, rs879922, rs233575, rs2074192, and rs2158083) in relation with anthropometric variables, and the plasma lipid and glucose profiles. Results: None of the five SNPs (rs4646188, rs879922, rs233575, rs2074192, and rs2158083) were significantly related with the variables studied in male adolescents. However, in girls, the occurrence of overweight or obesity was significantly associated with the presence of rs879922 (p=0,042), rs233575 (p=0,006), and rs2158083 (p=0,032). Also, the heterozygous genotypes of rs879922 (p=0.020), rs233575 (p=0.017) and rs2158083 (p=0.036) were significantly linked with the elevation of plasma triglycerides (TGs), and the two formers, with the increased TGs-HDL-C ratio. Moreover, the heterozygous genotypes of rs2158083 and rs2074192 were related with elevated total cholesterol (TC) (p=0.008 and p=0.003, respectively) and low-density lipoprotein cholesterol (LDL-C) (p=0.019 and p=0.03, respectively) concentrations. Conclusion: Mutations in ACE2 may promote cardiovascular risk factors. Moreover, the presence of rs879922, rs233575, rs2158083, and rs2074192 SNPs could be useful to predict overweight or obesity and higher levels of TGs and TC in females.*

26. **Author: Giovanna Castoldi;** Co Authors: G. Castoldi [1], R. Carletti [2], Silvia Ippolito [3], Andrea Stella [1], Gianpaolo Zerbini [4], Giovanni Zatti [1,5], Cira RT di Gioia [6]. Affiliations: [1].Dipartimento di Medicina e Chirurgia. Università degli Studi di Milano-Bicocca. Monza. Italy. (G.C., A.S.,G.Za.). [2].Dipartimento di Medicina Traslazionale e di Precisione. Sapienza Universita' di Roma. Roma. Italy. (R.C.). [3]. Laboratorio Analisi Chimico Cliniche. Ospedale San Gerardo. ASST Monza. Monza. Italy. (S.I.). [4]. Unita' Complicanze del Diabete. IRCCS Istituto Scientifico San Raffaele. Milano. Italy. (G.Ze.). 5. Clinica Ortopedica. Ospedale San Gerardo. ASST Monza. Monza. Italy. (G.Za.). [6]. Dipartimento di Scienze Radiologiche, Oncologiche e Anatomopatologiche. Istituto di Anatomia Patologica. Sapienza Universita' di Roma. Roma. Italy. (C.R.T.di G.). ***Title:*** *Cardioprotective effects of AT2 and Mas receptor activation in angiotensin II dependent hypertension. Background: The*

protective arm of the renin angiotensin system promotes cardioprotective effects. Aim: To investigate in experimental model of angiotensin II-dependent hypertension whether the activation of the AT2 or Mas receptors counteracts the onset of myocardial fibrosis and hypertrophy, and whether these effects are mediated by inflammatory mechanisms and/or sympathetic activity. Method: SD rats were treated for 4 weeks and divided in the following groups: a) Angiotensin II (Ang II,200 ng/kg/min, osmotic minipumps s.c); b) Ang II+Compound 21 (C21,0.3 mg/kg/day, i.p.); c) Ang II+Ang 1-7 (576microg/kg/day, i.p.); d) Ang II+Losartan (50mg/kg/day, p.o.); e) control group. Systolic blood pressure was measured by tail-cuff method. Myocardial fibrosis, hypertrophy, inflammatory cell infiltration and tyrosine hydroxylase expression, used as marker of sympathetic activity, were measured at the end of the protocol. Results: Ang II caused a significant increase of blood pressure, myocardial fibrosis and hypertrophy, as compared to control groups. C21 or Ang 1-7 administration did not modify the increase in blood pressure in Ang II treated rats, but both prevented the development of myocardial fibrosis and hypertrophy. Losartan blocked the onset of hypertension, myocardial fibrosis and hypertrophy in Ang II treated rats. C21 or Ang 1-7 or Losartan administration reduces myocardial inflammatory cell infiltration and tyrosine hydroxylase expression. Conclusion: In Ang II dependent hypertension the antifibrotic and antihypertrophic effects that follow the activation of the AT2 or Mas receptors are mediated by an antinflammatory mechanism and by a reduction of local sympathetic activity and are independent on the modulation of blood pressure.

27. **Author: Andrea Corbani;** Co Authors: G.E.M. Boari[1], F. Leidi[1,2], B. Accordini[1,2], F. Napoli, C. Ghidelli[1,2], G. Archenti[1,2], G. Gorla[1,2], B. Mangili[1,2], O. Scarano[1,2], D. Turini[1], M. Saottini[1], V. Guarinoni[1], G. Ferrari-Toninelli[1], F. Manzoni[1], D. Rizzoni[1,2]. Affiliations: [1]General Medicine, Hospital of Montichiari, Montichiari (BS), Italy; [2]University of Brescia, Brescia, Italy ***Title:*** *Comparison between first and second/third wave in moderate to severe COVID-19 in a hospital setting in Lombardy (Italy). INTRODUCTION We conducted a retrospective cohort study in COVID-19 Montichiari Hospital (Brescia, Italy). MATERIALS and METHODS 634 patients admitted in Medicine Ward with a moderate to severe COVID-19 were included in the present study. A group of 260 consecutive patients during SARS-CoV-2 from February to May 2020 and 374 consecutive patients from October 2020 to May 2021 were considered. Demographic data, comorbidities, ongoing treatment, and bio-humoral, respiratory, and haemodynamic data were recorded and compared. This study belongs to a wider one, aimed to assess long-term sequelae of moderate to severe COVID-19. RESULTS Main demographic data were not significantly different in the two considered time-lapses, except a lower prevalence of female sex during first wave. Mortality rate was significantly lower during the latter period (25% vs 10.9%; p<0.001). Time from symptoms onset to hospital admission was longer during first wave (8±6 vs 6±4 days; p<0.001) while hospital staying was significantly lower (10±14 vs 15±11 days; p<0.001), despite the well-known risk of prolonged hospital staying complications. Other significant differences were a wider use of corticosteroids and enhanced low-molecular weight heparin (LMWH) prophylactic dose as well as a minor antibiotic prescription during the second wave. No major differences between COVID-19 and hospital-staying related complications were detected. Ventilatory, bio-humoral and radiologic data were significantly poorer at the time of admission in first-wave patients. CONCLUSIONS On the base of preliminary data, a timely hospital admission is crucial; steroids are still a cornerstone in COVID-19 treatment while role of enhanced dose of LMWH remains controversial.*

28. **Author: M. Muñoz[1];** Co Authors: M.E. López-Oliva[1], E. Pinilla[1], C. Rodríguez[1], M.P. Martínez[2], J. Sáenz-Medina[3], C. Contreras[1], A. Sánchez[1], A. Gómez del Val[1], L. Rivera1 & D. Prieto[1]. Affiliations: [1]Department of Physiology and [2]Department of Anatomy and Embryology, Universidad Complutense de Madrid, Spain and [3] Department of Urology, Hospital Universitario Puerta de Hierro-Majadahonda, Madrid, Spain. ***Title:*** *Differential Contribution of Renal Cytochrome P450 Derivatives to Kidney Endothelial Dysfunction and Vascular Oxidative Stress in Obesity. Arachidonic acid (AA)-derived cytochrome P450 (CYP) metabolites, epoxyeicosatrienoic acids (EETs) and 20-*

hidroxyeicosatetranoic acid (20-HETE), play a key role in kidney metabolism and are involved in the regulation of both renal tubular and vascular functions and blood pressure (Fan & Roman, J Am Soc Nephrol 28:2845, 2017). Altered metabolism of CYP enzymes has differentially been involved in the pathogenesis of metabolic disease vascular complications. The present study aimed to assess whether obesity may induce changes in CYP2C and CYP4A enzymes and EETs- and 20-HETE production. Endothelial function and oxidative stress were assessed in intrarenal arteries of obese Zucker rats (OZR) and their lean counterparts lean Zucker rats (LZR) mounted and the effects of CYP2C and CYP4A inhibitors sulfaphenazole and HET0016, were examined on the endothelium-dependent relaxations and O2.- and H2O2 production in intact arteries. Non-nitric oxide (NO) non-prostanoid endothelium-derived hyperpolarization (EDH)-type responses were preserved but resistant to CYP2C9 blockade in OZR in contrast to those in LZR. Sulfaphenazole did not further inhibit reduced arterial H2O2 levels and CYP2C11 and CYP2C23 enzymes were down-regulated in intrarenal arteries from obese rats. CYP4A blockade restored impaired NO-mediated dilatation and reduced augmented O2.- production in kidney arteries from OZR. The current data demonstrate that both decreased endothelial CYP2C11/CYP2C23-derived vasodilator H2O2 and augmented CYP4A-derived 20-HETE contribute to endothelial dysfunction and vascular oxidative stress in obesity. CYP4A inhibitors ameliorate arterial oxidative stress and restore endothelial function which suggests its therapeutic potential for the vascular complications of obesity-associated kidney injury. This work was supported by PID2019-105689RB-I00 Spain.

29. **Author: Martina Cebova;** Co Authors: Andrej Barta, Olga Pechanova. Affiliations: Centre of Experimental Medicine, Institute of Normal and Pathological Physiology, Slovak Academy of Sciences. ***Title:*** *Effect of no donor, co donor and anti- hmgb1 protein in experimental myocardial infarction. Myocardial infarction (MI) followed by reperfusion triggers a complex sequence of pathophysiological responses. High mobility group box 1 (HMGB1) is a DNA-binding protein released during heart ischemia. Signaling molecules such as nitric oxide (NO) and carbon monoxide (CO) have a protective function and they reduce the risk of MI. The aim of the study was to compare the effect of Nicorandil as a NO donor, CORM-3 as a CO donor and nuclear protein HMGB-1 after experimentally induce MI in 12-week-old Wistar Kyoto rats. NOS activity was determined 7 days after surgery by conversion of 3[H] Arginine to 3[H] Citrulline in the ischemic, border and non-ischemic region of the heart. Endothelial (eNOS) and inducible NOS (iNOS) protein expression was determined by western blot analyses. Administration of Nicorandil, CORM-3 and anti-HMGB-1 protein into the myocardium before reperfusion increased the NOS activity in both ischemic and border part of the heart. The same pattern was observed in the eNOS expression after anti-HMGB-1 as well as Nicorandil administration in the border and non-ischemic region of the heart. The CORM-3 decreased the eNOS expression in ischemic and border zone. The iNOS expression was decreased in the non-ischemic part after administration of all substances. Anti-HMGB-1 protein administration decreased iNOS expression in the ischemic zone as well as TNF-alpha and IL-6 level in plasma. Considering the results, NO and CO donors as well as anti-HMGB-1 protein are promising molecules for reduction the negative effects of the myocardium infarction, as well as for the treatment of cardiovascular diseases.*

30. **Author: Carme Ballester-Servera;** Co Authors: C. Ballester-Servera, L. Cañes, J. Alonso, S. Aguiló, C. Rodríguez, J. Martínez-González. Affiliations: Instituto de Investigaciones Biomédicas de Barcelona (IIBB-CSIC), Institut de Recerca Hospital de la Santa Creu i Sant Pau, IIB-Sant Pau, CIBERCV. ***Title:*** *High vascular expression of Lysyl Oxidase (LOX) exacerbates atherosclerosis and vascular calcification. Background: Vascular calcification is associated to an active remodeling of the extracellular matrix (ECM). Vascular smooth muscle cells (VSMC) from transgenic mice overexpressing the ECM-modifying enzyme LOX (TgLOX) exhibited increased calcification and osteoblast commitment under osteogenic conditions. Aim: Our objective was to assess the impact of the specific overexpression of LOX in VSMC on vascular calcification associated to atherosclerosis.*

Methods: Hyperlipidemia and atherosclerosis was induced by an adeno-associated virus vector that stably express a gain-of-function mutant form of PCSK9 in the liver (AAV- PCSK9D374Y), and a high-fat diet. Atherosclerosis in the innominate artery was monitored by ultrasounds. Body weight, plasma levels of cholesterol, triglycerides and glucose were analyzed. The hepatic expression of the LDL receptor (LDLR) was analyzed by immunoblot. En face preparations of the aorta were used to quantify the extent of atherosclerosis. Vascular calcification and collagen content was assessed by von Kossa and picosirius red staining, respectively, and gene expression by RT-PCR. Results: AAV-PCSK9D374Y strongly inhibited the expression of the LDLR in the liver. LOX transgenesis slightly increased the extent of atherosclerosis, in particular in abdominal aorta, and significantly increased vascular calcification (approx. 1.7 to 4.7-fold respect to WT animals depending on the vascular bed). Plaques from TgLOX mice exhibited a higher content of mature collagen. Key genes involved in calcification were upregulated in atherosclerotic aortas, but no significant differences were observed between TgLOX and WT mice. Conclusion: our findings highlight the relevance of ECM processing by LOX in vascular calcification in atherosclerosis.

31. **Author: Juan Pablo González Appelgren ;** Co Authors: R. Caulier, JE. Oyarzún1, A. Eblen-Zajjur, S. Uribe. Affiliations: Center for Biomedical Imaging, Pontificia Universidad Católica de Chile; Millennium Nucleus for Cardiovascular Magnetic Resonance, Santiago, Chile. ***Title:*** *Peri-spinal neurovascular response triggered by a painless peripheral stimulus in patients with chronic high blood pressure. Background. Chronic High blood pressure (CHBP) is associated with reduced pain perception, known as BP-related hypoalgesia. Despite clinical implications in cardiovascular risk groups, the association between CHBP and nociception is not well understood. Current medical science lacks an effective test of the somatosensory and nociception functions of the spinal cord. Objective. The aim of this study was to measure the peri-spinal neurovascular response (NVR) at different spine levels in 22 CHBP and in 42 healthy volunteers. Methods. Our research group described a non-invasive recording of the peri-spinal NVR, triggered by electrical stimulation of a peripheral nerve, using an 8 channel functional near infrared spectroscopy (fNIRS) device. Results. NVR in cervical region of healthy group shows rise time 12.27±4.41 (s); Amplitude 0.00026±0.00014 (a.u.) and duration 15.34±3.6 (s) these values were compared to CHBP group shows rise time 13.72±3.84 (p=0.056); Amplitude 0.00011±0.00009 (p=0.000046) and duration 14.17±5.06 (p=0.2) by non-parametric Mann-Whitney test. Discussion. This study shows preliminary findings about differences between CHBP compared to healthy subjects in the peri-spinal NRV recorded by fNIRS which strongly support the notion for an altered excitability of the neuronal population and/or the neurovascular coupling of the spinal dorsal horn. Conclusions. Future applications of this method would give relevant clinical information about evolution and response to the treatment. [242 words] Funded by FONDEF grant Number ID18I0064, Fondecyt 1181057 and the Millennium Nucleus on Cardiovascular Magnetic Resonance of the Millennium Science Initiative, from the National Agency for Research and Development, ANID; and Fundación COPEC-UC grant Number 2018R.1030.*

32. **Author: Maria Piazza[1,2,3];** Co Authors: N.M.J Hanssen[2,4], J Scheijen[1,2], M. vd Waarenburg[1,2], B. Caroccia[3], T.M. Seccia[3], C.D.A. Stehouwer[1,2], G.P. Rossi[3], C.G. Schalkwijk[1,2]. Affiliations: [1.] Department of Internal Medicine, Maastricht University Medical Centre, Netherlands, [2.] Cardiovascular Research Institute Maastricht (CARIM), Netherlands, [3.] Department of Medicine-DIMED University of Padua, Italy, [4.] Department of Vascular and Internal Medicine, Amsterdam University Medical Center. ***Title****: Serum dicarbonyl and AGE levels are not associated with levels of AT1AA in patients with aldosterone-producing adenoma. Background Patients with aldosterone-producing adenoma (APA) have higher risk of cardiovascular disease and high levels of autoantibodies (AT1AA) against the angiotensin II type I receptor (AT1R). AT1R activation is linked to an increase of glucose metabolite methylglyoxal (MGO), a potential precursor of advanced glycation endproducts (AGEs) and a driver of cardiovascular disease. Aim To investigate whether serum AT1AA levels are associated with serum MGO and AGE levels in APA patients. Methods In 26*

patients with APA (51±7.7y) we measured by ultra-performance liquid chromatography tandem mass-spectrometry the serum levels of dicarbonyls MGO, glyoxal (GO) and 3-deoxyglucosone (3-DG), as well as the major dicarbonyl-derived AGEs 5-hydro-5-methylimidazolone (MG-H1), Nε-(carboxyethyl)lysine (CEL) and Nε-(carboxymethyl)lysine (CML). AT1AA were measured by ELISA and in a subset of 14 patients, these measurements were repeated 1 month after adrenalectomy. Results Baseline AT1AA levels showed no association with baseline serum MGO, GO and 3-DG, or protein-bound and free MG-H1, CEL and CML levels. No changes were observed in any of the dicarbonyl and protein-bound AGE levels after adrenalectomy. However, there was a significant increase of free CML, CEL and MG-H1 levels (CML from 86 nmol/L to 142 nmol/L, CEL from 46 nmol/L to 63 nmol/L and MG-H1 from 110 nmol/L to 237 nmol/L; all p<0.05)). Conclusion The elevated serum levels of AT1AA are not associated with serum dicarbonyls, or protein-bound and free AGE levels in patients with APA. Serum free AGEs level were higher after adrenalectomy, potentially due to changes in kidney function or use of less medications after surgery.

33. **Author: Lucía Serrano;** Co Authors: A. B. García-Redondo, M. Salaices, A. M. Briones. Affiliations: Pharmacology and Therapeutics Department, Faculty of Medicine, University Autónoma, Madrid, Spain. ***Title:*** *Fat-1 transgenic mice are protected against vascular damage in hypertension. Background: Vascular functional and structural alterations induced by hypertension are influenced by low-grade chronic inflammation. Resolution of inflammation is mediated by specialized pro-resolving mediators (SPMs), which derive from n3 fatty acids (PUFAs). Previous evidence suggest that SPM prevent vascular damage in several pathological situations. The transgenic fat-1 mice converts n6 into n3 fatty acids, resulting in greater level of anti-inflammatory mediators in their tissues. Aim: To evaluate the effects of Angiotensin II (AngII) in blood pressure and vascular damage in fat-1 mice. Methods: Aorta, mesenteric resistance arteries (MRA) and perivascular adipose tissue (PVAT) were taken from heterozygous fat-1 mice and its corresponding WT littermates infused or not with AngII. Blood pressure was measured by tail-cuff plethysmography. Vascular function and structure were studied with wire and pressure myographs and confocal microscopy. Gene and protein expression were analyzed with RT-PCR and cytokine array. Results: Fat-1 mice were partially protected against the AngII-induced increase in blood pressure. Moreover, AngII induced endothelial dysfunction in aorta and MRA from WT but not in fat-1 mice. Similarly, AngII increased vascular contractile response in aortas from WT but this hypercontractility was prevented in fat-1 genotype. Fat-1 mice were also protected against vascular stiffness, possibly by attenuation of AngII-induced decrease in fenestrae size in the internal elastic lamina. In addition, fat-1 mice showed reduced AngII-induced macrophages infiltration in PVAT and decreased inflammation in aorta. Conclusion: Our data shows that fat-1 mice are protected from the vascular alterations induced by hypertension likely through a reduction in inflammatory mediators.*

Poster Session 2

34. **Author: Giulia Gorla;** Co Authors: G.E.M. Boari[1], O. Scarano[1,2], B. Mangili[1,2], A. Corbani[1,2], F. Leidi[1,2], B. Accordini[1,2], F. Napoli, C. Ghidelli[1,2], G. Archenti[1,2], D. Turini[1], M. Saottini[1], V. Guarinoni[1], G. Ferrari-Toninelli[1], F. Manzoni[1], A. Marengoni[2], D. Rizzoni[1,2]. Affiliations: [1]General Medicine, Hospital of Montichiari, Montichiari (BS), Italy; [2]University of Brescia, Brescia, Italy. ***Title:*** *Correct timing in hospital admission impacts on outcome of COVID-19 elderly patients: a comparison between first and second wave. INTRODUCTION Coronavirus disease 2019 (COVID-19) outbreak represented a major clinical problem in Lombardy, one of the most affected Italian (and worldwide) Regions, in terms of death toll and long-term sequelae. This is particularly true when elder people are taken into account. MATERIALS and METHODS In the present study we retrospectively recorded data of elderly patients (> 65 years) admitted to General Medicine ward (COVID-19 M unit) of the Montichiari Hospital (Brescia, Italy) during COVID-19 first (March-May 2020) and second/third wave (October 2020-May 2021) and compared them to better understand optimal*

approach to COVID-19 elderly patients. RESULTS A total of 407 patients older than 65 were treated: 185 of them during first wave and 222 during second/third wave. Of them, 63 died during first wave and 36 during second wave. Mortality rate was significantly lower during the latter period, while there was almost no difference in age, comorbidities and ongoing treatment. When comparing number and type of complications we found no significant differences. The two groups were mainly different for the quicker hospital admission, confirmed by a less severe bio-humoral, haemodynamic ed radiologic profile and a wider use of steroid treatment. Other minor differences did not explain mortality reduction in elderly patients (i.e. different doses of low molecular weight heparin or antibiotic therapy). CONCLUSIONS Our data suggest the importance of a rapid hospital admission of elderly patients who show a quickly worsening of their condition; steroids are confirmed as a cornerstone in moderate to severe COVID-19 treatment.

35. **Author: I. Solchaga-Sánchez[1];** Co Authors: J.J. Gómez-de Diego[2], P. Rodríguez-Rodríguez[3], D. Muñoz[3], M.J. Delgado[4], B. Quintana-Villamandos[5]. Affiliations: [1]Anaesthesiology and Intensive Care, Hospital Gómez Ulla, Madrid, Spain. [2]Department of Cardiology, Hospital Clínico San Carlos, Madrid, Spain. [3]Faculty of Medicine, Universidad Autonoma de Madrid, Spain. [4]Department of Experimental Medicine and Surgery, Health Research Institute of Hospital Gregorio Marañón, Madrid, Spain. [5]Anaesthesiology and Intensive Care, Hospital Gregorio Marañón, Madrid, Spain. ***Title:*** *Long-term effect of esmolol on the left ventricle structure in an experimental model of ventricular hypertrophy. Background: Hypertension has a high prevalence worldwide, leading to left ventricular hypertrophy (LVH) without treatment. Our group has demonstrated the regression of LVH after 48h of treatment with esmolol in an experimental model of primary arterial hypertension and LVH (SHR, spontaneously hypertensive rat). Aim of the study: to assess the continuity of short-term esmolol therapy effect about regression of LVH. Materials and Methods: fourteen-month-old male SHRs were treated with intravenous perfusion of esmolol (SHR-E) at 300 μg/kg/min for 48h or saline as vehicle (SHR). At the end of the treatment, a week and a month after the administration, systolic arterial pressure, heart rate and left ventricle mass were assessed using transthoracic echocardiography (M mode); left ventricular biopsies were taken to analyse the size of cardiomyocytes (cross sectional area) and the collagen volume fraction as a marker of fibrosis. P< 0.05 was considered significant. All procedures were approved by the Ethics Committee of our institution. Results: Esmolol produces a decrease in blood pressure, heart rate and left ventricular mass index (LVMI) in SHR-E (P<0,05) after 48h of treatment; the histological analysis shows statistically significant lower values in the cross sectional area of cardiomyocytes and collagen volume fraction in SHR-E compared to SHR. These results remains one week and one month later. Conclusion: short-term treatment with esmolol reversed LVH in SHR rats and these positive changes stay over time. Further studies are needed to confirm these results and apply them to humans in the future.*

36. **Author: Jenaro Espitia-Corredor [1,2]**; Co Authors: L. Shamoon [1]; C. Rimassa-Taré [2]; C. Sánchez-Ferrer [1,3]; C. Peiró [1,3]; G. Díaz-Araya [2,4]. Affiliations: [1.] Department of Pharmacology, Faculty of Medicine, Universidad Autónoma de Madrid, Madrid, Spain. [2]. Laboratorio de Farmacología Molecular (FARMOLAB), Department of Pharmaceutical and Toxicological Chemistry, Faculty of Chemical Sciences and Pharmacy, Universidad de Chile, Santiago, Chile. [3]. Instituto de Investigación Sanitaria del Hospital Universitario La Paz (IdiPAZ), Madrid, Spain. [4.] Advanced Center for Chronic diseases ACCDiS, Universidad de Chile, Santiago, Chile. ***Title****: Resolvin E1 attenuates doxorubicin- and interleukin-1beta-induced cardiac fibroblast senescence. Background Aging is characterized by a loss of physiological integrity and increased vulnerability to diseases such as hypertension and heart failure. Cardiac fibroblasts (CF) participate in cardiovascular aging, by creating a damage-associated pro-inflammatory state. Recent studies suggested that chemotherapeutic drugs -doxorubicin (Doxo)- or pro-inflammatory molecules -interleukin-1beta (IL-1b)- promote cardiovascular aging. Few studies have evaluated the effect of Doxo or IL-1b on CF senescence. There is a need to search for CF senescence inhibitors as therapeutic tools for retarding age-related cardiac*

diseases. The pharmacological potential of the pro-resolving lipid mediators (SPM) of inflammation as anti-senescence agents in CF has not been tested yet. Objective We investigated whether Doxo and IL-1b induce senescence in cultured adult mouse cardiac fibroblast (AmCF), as models of pro-inflammatory cardiac damage. The ability of resolvin E1 (RvE1) - an endogenous SPM - to prevent Doxo- and IL-1b-induced CF senescence, was also evaluated. Methods CF from adult C57BL/6 male mice by collagenase digestion. Senescent cells were identified by senescence-associated-β-galactosidase (SABG) staining. p53, p21, and gammaH2A.X were determined via western blot. Results Both Doxo (10 nM) and IL-1b (2.5 ng/mL) augmented the number of SABG positive cells and the levels of senescence markers p53, p21, and gammaH2A.X at 24h post stimuli without affecting cell viability. Moreover, RvE1 (100 nM) was capable to reduce Doxo- and IL-1b-induced upregulation of CF senescence markers. Conclusion RvE1 may have a protective role against CF senescence, offering a novel therapeutic approach to prevent age-related cardiac disorders.

37. **Author: Constanza Ballesteros-Martinez;** Co Authors: Raquel Rodrigues-Diez, Ernesto Martínez-Martínez, Luis Beltrán, María Gonzalez-Amor, Victoria Cachofeiro, Mercedes Salaices, Ana M. Briones. Affiliations: Department of Pharmacology, Faculty of Medicine, Universidad Autónoma de Madrid, Hospital La Paz Institute for Health Research, Madrid, CiberCV, Spain. ***Title:*** *Role of Microsomal Prostaglandin E Synthase-1 (mPGES-1) in the metabolic, cardiovascular and renal alterations associated with obesity. Background: Obesity is a risk factor for the development of metabolic and cardiovascular alterations. Microsomal prostaglandin E synthase 1 (mPGES-1) is responsible for the production of prostaglandin E2 (PGE2) under inflammatory conditions. PGE2 is a key lipid mediator that participates in vascular damage associated to inflammatory processes. Objective: This study evaluates the role of mPGES-1 in the development of metabolic and cardiovascular alterations associated with obesity. Methods: We developed a model of high-fat diet (HFD, 60% fat)-induced obesity in male mPGES-1-/- and mPGES-1+/+ mice. The glycaemic profile was studied by glucose and insulin tolerance tests. Vascular function, and structural and mechanical properties of aorta and mesenteric resistance arteries (MRAs) were evaluated by isometric and perfusion myographs. Histological studies, q-RT-PCR and Western Blot analyses were performed. Gene expression in abdominal fat from patients and its correlation with vascular damage was determined. Results: Our results show that mPGES-1-/- mice fed with HFD are protected against body weight gain and present better glycaemic profile compared to mPGES-1+/+ mice. At cardiovascular level mPGES-1-/- mice are protected against vascular functional and structural alterations, vascular remodelling and inflammation, and cardiac hypertrophy and fibrosis induced by HFD. Moreover, mPGES-1-/- mice are protected against renal fibrosis and inflammation, and glomerular remodelling. In patients, mPGES-1 expression in abdominal fat positively correlates with vascular remodelling and stiffness, and with systolic blood pressure. Conclusion: Our data suggest that mPGES-1 could be a novel therapeutic target to prevent some of the metabolic, renal and cardiovascular alterations associated with obesity.*

38. **Author: Daniel González Moreno;** Co Authors: Elena Vega-Martín, Marta Sanz Gómez, Francisco J. Manzano-Lista, Marta Gil-Ortega, Beatriz Somoza, Reinhold Kreutz, María S. Fernández-Alfonso. Affiliations: Instituto Pluridisciplinar/Complutense University. ***Title:*** *The diabetic Munich Wistar Frömter Rat: a new model of diabetic nephropathy. Background: Diabetic nephropathy (DN), one of the most common complications of diabetes, is associated with cardiovascular (CV) disease. The development of new pharmacological therapies for the treatment of DN-related CV limited by the lack of appropriate DN-animal models reflecting human DN. Aims: To develop an animal model of DN through the induction of diabetes in the Munich Wistar Frömter (MWF) rat, a genetic model of chronic kidney disease. Methods: 16-week-old male MWF rats were fed a standard (MWF-C) or a high-fat/high-sucrose (HF/HS) diet for 6 weeks together with streptozotocin (STZ; 15mg/Kg) at the start of diet exposure (MWF/STZ/HF/HS). Additionally, MWF-C rats were treated with STZ and diary injected with insulin (MWF/STZ/INS). Wistar rats were used as control. N=7 rats/group. Haemodynamic*

parameters were analysed by direct cannulation in both the carotid and the femoral artery. A glucose tolerance test (GTT) was performed a week before sacrifice. Results and discussion: Water intake, urine excretion, GTT and HOMA index evidenced the development of diabetes in MWF/STZ/HF/HS rats. The expression of KIM-1 and NGAL, markers of renal damage, were more than 10-fold higher in MWF/STZ/HF/HS rats than in MWF-C. Insulin injection normalized those parameters. Albuminuria was higher in MWF-C with no further effect of diabetes. Both systolic, diastolic blood pressure, and pulse wave velocity were higher in MWF-C rats compared with Wistar rats but unmodified by the induction of diabetes Conclusion: The MWF/STZ/HF/HS model has shown to develop both diabetes and further kidney damage compared with MWF and might constitute a promising model for DN.

39. **Author: Carlo Barsali**; Co Authors: Carlo Barsali, Armando Ferrera, Alberto Michielon, Gaetano Marino, Massimo Volpe, Carmine Savoia. Affiliations: Clinical and Molecular Medicne Department, Cardiology Unit, Sant'Andrea Hospital, Sapienza University of Rome, Rome, Italy. ***Title:*** *The impact of Transcatheter Aortic Valve Implantation (TAVI) on the function of resistance arteries in patients with severe aortic stenosis. Background: Patients with severe aortic stenosis (AS) showed adaptation of the vascular stiffness of conductance arteries after TAVI. The role of early adaptation of peripheral arterial resistance after TAVI remains to be evaluated. AIM: We studied the parameters of early functional adaptations of resistance arteries by pulse wave analysis (PWA) assessed by applanation tonometry. Methods: 21 patients (age 81,43±0,75 years, 48% male, 52% female) were studied before and 48 hours after TAVI. Patients had hypertension (100%), diabetes (19%), dyslipydemia (29%) and were on therapy with: beta-blockers (62%), diuretics (52%), RAS blockers (90%), calcium-channel blockers (33%), statins (29%), oral hypoglycaemic agents (19%). By applanation tonometry (Sphygmocor) we evaluated: 1) ejection duration (ED); 2) parameters of aortic stiffness: pulse wave velocity (PWV) and pulse pressure (PP); 3) parameters of functional adaptation of peripheral vascular resistance: central (cAI@75%) and peripheral (pAI@75%) augmentation index, reflection index (RI). Peripheral and central systolic/diastolic blood pressure (BP) was measured by oscillometric device and tonometry respectively. By echocardiography we evaluated aortic size (bulb and ascending aorta), aortic transvalvular gradient and parameters of systolic/diastolic left ventricular function. Results: TAVI reduced transvalvular gradient (8,05±1,83 vs 46,14±2,57 mmHg, p<0,0001) and ED (296,8±7,06 vs 333,3±4,85 ms, p<0,001). Furthermore, TAVI reduced cAI@75 (27,35±1,73 vs 35,38±1,83%, p<0,05), pAI@75 (-12,57±4,42 vs -1,46±2,54%, p<0,05), and RI (67,27±4,19% vs 80,93±3,24%, p<0,05). BP, aortic size, systolic/diastolic ventricular function, PWV, and PP remained unchanged after TAVI. Conclusions: PWA showed that early hemodynamic adaptations in patients with severe AS after TAVI are characterized by a significant vasodilation in resitance arteries.*

40. **Author: Marta Sanz-Gómez;** Co Authors: S. Quesada[2], L. Lagartera[2], M. Beroiz[1], J. Cumella[2], C. Pérez[2], A. Castro[2], MS. Fernández-Alfonso[1]. Affiliations: [1]. Instituto Pluridisciplinar and Facultad de Farmacia, Universidad Complutense de Madrid (UCM), Spain [2]. Instituto de Química Médica del Consejo Superior de Investigaciones Científicas (IQM-CSIC), Madrid, Spain. ***Title:*** *The spiranic compound SPI3 activates the endothelial AMPK-eNOS pathway leading to a nitric oxide-dependent vasodilation. Background and objectives: One of the main metabolic syndrome-associated complications is endothelial dysfunction, that leads to the development of hypertension and cardiovascular (CV) disease, due to an inactive AMPK-eNOS pathway and the lack of endothelial nitric oxide (NO) release. Our group has developed a new family of spiranic AMPK modulators to trigger AMPK activation in the search of pharmacological candidates for the prevention and/or treatment of the metabolic syndrome-associated CV damage. Methods: Binding of these new compounds to the human endothelial AMPK isoform, AMPKα1β1γ1, was performed by Surface Plasmon Resonance (SPR). The activity of the best-binding compound was assessed in human endothelial cells (EAHy926) by Western Blot and fluorescence microscopy, as well as with rat aortic function. Results: From all spiranic compounds binding AMPKα1β1γ1, SPI3 was selected as the best one (11.8 RU). Treatment of EAHy926 with SPI3 (1, 10 and 100μM) enhanced p-Thr174AMPK levels,*

which translates to phosphorylation of both Ser79ACC and Ser1177eNOS (1 and 10 µM), as well as to NO increase (5 µM). The SPI3-induced activation was similar to the stimulation promoted by both AICAR (5mM) and 2-DG (1mM). Furthermore, SPI3 elicited a concentration-dependent vasodilation (1nM to 100µM) comparable to the one reached with the AMPK activator, A-769662, at the same concentrations. Conclusion: SPI3 is a medium-potency endothelial AMPK activator that also promotes a vasodilation in rat aorta. We suggest that it might be a candidate for further investigation as a possible pharmacological treatment for endothelial dysfunction and hypertension associated to metabolic syndrome.

41. **Author: Kendal Ragusky;** Co Authors: Pilar Rodriguez-Rodriguez (PhD), Sophida Puthong (PhD), Santiago Ruvira (Master Degree), Silvia Cañas (Master Degree), Miguel Rebollo-Hernanz (PhD), M Angeles Martín-Cabrejas (PhD) and Silvia M. Arribas (PhD). Affiliations: FOSCH group. Departments of Physiology (Faculty of Medicine) and CIAL (Center for Food Research) from Universidad Autónoma de Madrid. ***Title:*** *Vasoactive Properties of Cocoa Shell waste-product, a potential nutraceutical for cardiovascular disease. Cocoa consumption has hypotensive and antioxidant effects, related to its content in phenolic compounds and methylxanthines. Cocoa manufacturing releases enormous amounts of waste to the environment. We propose that some of its by-products with similar composition may be used to developed nutraceuticals for cardiovascular disease. Our aim was to investigate the vascular properties and the mechanism of an extract procured from the cocoa bean shell (CCE), an unused waste product, as well as those of its primary components: caffeine (C), protocatechuic acid (PCA) and theobromine (T). Iliac arteries from young and aged Sprague-Dawley male and female rats were used. Vascular responses were assessed through isometric tension experiments and DHE (dye interacting with O2) and confocal microscopy was used to determine O2- scavenging capacity. In noradrenaline pre-contracted arteries CCE caused vasorelaxation, irrespective of age and sex. Compared to ACh, CCE maximal response and EC50 were significantly lower iliac (Ach EC50= 7,76 10-8 M; CCE EC50= 4,9 10-9 M). CCE vasodilatory effect was endothelium-dependent and was abolished by L-NAME, but not by indomethacin. CCE incubation significantly reduced the number of DHE-positive cells. Vasodilatation was significantly larger in arteries from male aged rats compared to female counterparts (male=-53.55±8.2%; female-20.67±4.6 %). The main components C, T and PCA also demonstrated antioxidant and endothelium-dependent vasodilatory effects. In conclusion, CCE has in vitro vasodilatory properties, present at low concentrations, linked to preservation of NO bioavailability through O2-. scavenging capacity of phenolic compounds and methylxanthines. CCE can be used to develop a nutraceutical to mitigate cardiovascular diseases.*

42. **Author: Daniele Teixeira Alves;** Co Authors: L. F. Mendes; W. O. Sampaio; L. M. C. C. Campos; M. A. R. Vieira; A J. Ferreira; A. S. Martins; E. Popova; M. Todiras; F. Qadri; N. Alenina; M. Bader; R. A. S. Santos; M. J. Campagnole-Santos. Affiliations: Max- Delbrück Center for Molecular Medicine-MDC, Berlin, Germany; DZHK (German Centre for Cardiovascular Research), Partner Site Berlin, German. ***Title:*** *Hemodynamic phenotyping of transgenic rats with ubiquitous expression of an angiotensin-(1-7)-producing fusion protein. Background: Activation of the angiotensin (Ang) converting enzyme 2/Ang-(1-7)/MAS pathway of the renin-angiotensin system induces protective mechanisms in different diseases. Aim: we describe the cardiovascular phenotype of a new transgenic rat line (TG7371) that expresses an Ang-(1-7)-producing fusion protein. Methods and Results: Transgene mRNA and the protein were shown to be present in all evaluated tissues of TG7371 with the highest expression in aorta and brain. Plasma Ang-(1-7) levels, measured by radioimmunoassay were similar to control SD rats. TG7371 showed lower baseline mean arterial pressure, assessed in conscious or anesthetized rats by telemetry or short-term recordings, associated with increased plasma ANP and higher urinary sodium concentration. Evaluation of regional blood flow and hemodynamic parameters with fluorescent microspheres showed a significant increase in blood flow in different tissues (kidneys, mesentery, muscle, spleen, brown fat, heart and skin), with a resulting*

decreased total peripheral resistance. TG7371 rats also presented increased cardiac and global sympathetic tone, increased plasma AVP levels and decreased free water clearance. Conclusion: Altogether, our data show that expression of an Ang-(1-7)-producing fusion protein induced a hypotensive phenotype due to widespread vasodilation and consequent fall in peripheral resistance. This phenotype was associated with an increase in ANP together with an increase in AVP and sympathetic drive, which did not fully compensate the lower BP. Here we present the hemodynamic impact of long-term increase in tissue expression of an Ang-(1-7)-fusion protein and provide a new tool to investigate this peptide in different pathophysiological conditions.

43. **Author: Berta Ganizada;** Co Authors: S. Parikh, M. Ramaekers, A.C. Akbulut, I. Cortenraad, F. Kerckhove, C. Willems, G. Debeij, E. Natour, R. Lorusso, J. Wildberger, J.G. Maessen, R. Accord, S. Schalla, K. Reesink, L. Schurgers, E. Bidar. Affiliations: Cardio Thoracic Surgery/ Faculty of Health, Medicine and Life Sciences/School of Cardiovascular Diseases/Maastricht University Medical Center. ***Title:*** *Prospective Biobank for Ascending Thoracic Aortic Aneurysm Research: Integrating Clinical, Imaging and Pathophysiology Perspectives. Background: For patients with an ascending thoracic aortic aneurysm (aTAA), treatment decision heavily depends on imaging diameters or growth rate, whereas the variability in characteristics is considerable among surgical repair patients [1]. Integrity of aortic tissue is subject to homeostatic mechanisms, centering around the interaction between the extracellular matrix and cells, in particular the vascular smooth muscle cells (VSMCs) [2]. Recent literature review indicates that most studies do not consider the relationships between clinical, imaging and tissue-cell characteristics. Hence, we aim to set-up a prospective biobank and infrastructure to exploit these comprehensive data. Methods: The biobank was approved by our institutional ethics committee. Patients receive MRI examination in routine pre-operative work-up, including 4D-flow [3]. After surgical resection, the aneurysm tissue is dissected along 4 circumferential and 2 longitudinal orientations. Per-operative video-tracking measurements of local mechanical strains [4] as well as tissue sampling are then mapped onto MRI aneurysm geometry. Samples are processed and stored for immunohisto/cytochemical analyses, wherein we focus on VSMC phenotype, MMP expression, calcification and inflammatory markers [2]. Our current target research question is: how do local differences in wall strain as well as fluid shear stress correlate to differences in tissue and VSMC markers at anatomically corresponding locations? Results: Table 1 summarizes the aTAA patient cohort included between July 2019 and September 2021. Our target is inclusion of at least 200 cases by the end of 2027. Conclusion: We have established a multi-aspect infrastructure for research into ascending thoracic aortic aneurysm, facilitating mechanistic research.*

44. **Author: Yong Wang;** Co Authors: M. Kalhöfer-Köchling, Y.X. Zhang, M. Uecker, S. Boretius, W.-H. Zimmermann, E. Bodenschatz. Affiliations: Laboratory for Fluid Physics, Pattern Formation and Biocomplexity, Max Planck Institute for Dynamics and Self-Organization, 37077 Göttingen, Germany. ***Title:*** *In Silico Modeling for Tissue Engineered Heart Repair. Background: Heart failure is a fatal disease with reduced pump function. Its common cause is myocardial damage with insufficient endogenous capacity for cardiac regeneration. Current options for patients with end-stage heart failure include mechanical support devices and heart transplants. Engineered heart muscle (EHM) may offer new avenues to repair the failing heart. Objective: We use in silico modeling to study cardiac mechanics and guide new therapies such as tissue engineered heart repair. Methods: Using nonlinear solid mechanics, computer modeling and medical images, we developed a heart model for EHM implantation. Since we focus on the pump function of the heart, only the left ventricle (LV) was considered, whose geometry can be idealized as a truncated ellipsoid or reconstructed from MRI/CT scan data. The myocardium was considered as an orthotropic elastic material whose stress-strain relationship is defined by a constitutive law. To achieve better physiological agreement and smoother simulations, we took fiber dispersion into account and proposed a new class of constitutive laws. The infarcted heart with and without EHM were simulated. Results and Discussion: In silico experiments were performed for an idealized left ventricle. Different types of EHM configurations were considered.*

Numerical results show that EHM can improve the pump function of the LV and thus may be used to repair the failing heart. We also numerically tested the EHM with realistic geometry of the LV. We propose that our in silico model can inform the EHM construction process allowing for a personalized EHM design and its optimal surgical placement.

45. **Author: Lorenzo Carnevale;** Co Authors: Raimondo Carnevale, Francesco Mastroiacovo, Marialuisa Perrotta, Daniela Carnevale, Giuseppe Lembo. Affiliations: Research Unit of Neuro and Cardiovascular Pathophysiology, IRCCS Neuromed and Sapienza University of Rome. ***Title:*** *Advanced brain injury characterization by neuroimaging in a mouse model of hypertension-induced cognitive declineBackground: COVID-19 association with cardiovascular disease is thought to be due to endothelial cell inflammation. ACE2 interactions with SARS-CoV-2 spike protein S1 subunit is important to viral infection. Aims: Here we questioned whether SARS-CoV-2 induces vascular inflammation via ACE2 and whether this is related to viral infection. Methods: Human microvascular endothelial cells (EC) were exposed to recombinant S1p (rS1p) 0.66 μg/mL for 10 min, 5h and 24h. Gene expression was assessed by RT-PCR and levels of IL6 and MCP1, as well as ACE2 activity, were assessed by ELISA. Expression of ICAM1 and PAI1 was assessed by immunoblotting. ACE2 activity was blocked by MLN4760 (ACE2 inhibitor) and siRNA. Viral infection was assessed by exposing Vero E6 (kidney epithelial cells; pos ctl) and EC to 105 pfu of SARS-CoV-2 where virus titre was measured by plaque assay. Results: rS1p increased IL6 mRNA (14.2±2.1 vs. C:0.61±0.03 2^-ddCT) and levels (1221.2±18.3 vs. C:22.77±3.2 pg/mL); MCP1 mRNA (5.55±0.62 vs. C:0.65±0.04 2^-ddCT) and levels (1110±13.33 vs. C:876.9±33.4 pg/mL); ICAM1 (17.7±3.1 vs. C:3.9±0.4 AU) and PAI1 (5.6±0.7 vs. C: 2.9±0.2), p<0.05. MLN4760, but not rS1p, decreased ACE2 activity (367.4±18 vs. C: 1011±268 RFU, p<0.05) and blocked rS1p effects on ICAM1 and PAI1. ACE2 siRNA blocked rS1p-induced IL6 release, ICAM1, and PAI1 responses as well as rS1p-induced NFκB activation. EC were not susceptible to SARS-CoV-2 infection, while the virus replicated well in Vero E6. Conclusion: In conclusion, rS1p induces an inflammatory response through ACE2 in endothelial cells; an effect that was independent of viral infection.*