BELGIAN SOCIETY OF

PHYSIOLOGY AND PHARMACOLOGY

NATIONAL COMMITTEE OF PHYSIOLOGY AND PHARMACOLOGY

—

Autumn Meeting

Friday, November 25th 2022

PROGRAMME

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Venue

Palace of the Academies
Royal Academy of Medicine of Belgium
"Rubens room"
Rue Ducale / Hertogstraat 1
1000 Brussels

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Local host

Prof. Dr. Lynn Roth
Laboratory of Physiopharmacology
UAntwerpen
Belgium

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with support of the

Royal Flemish Academy of Belgium for Science and the Arts

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Venue

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"Rubens room"
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1000 Brussels

09.15-10.00 Welcome with coffee and tea

Keynote lecture

10.00-11.00 **Arterial stiffness: More than just a number**
Bart Spronck (Maastricht University, Biomedical Technology, School for Cardiovascular Diseases)

Oral communications (morning session)

11.00-11.15 **High Pulsatile Load Reversibly Inhibits Aortic FAK Activity And Decreases Viscoelasticity**
Cédric H.G. Neutel, Callan D. Wesley, Guido R.Y. De Meyer, Wim Martinet, Pieter-Jan Guns (UAntwerp)

11.15-11.30 **Arterial Stiffness Is Transient During Doxorubicin Treatment Despite Persistent Endothelial Cell Dysfunction: a Longitudinal Study Complemented by Proteomics**
Matthias Bosman, Dustin N. Krüger, Charles H. Van Assche, Cédric H.G. Neutel, Kasper Favere, Constantijn Franssen, Wim Martinet, Lynn Roth, Guido R.Y. De Meyer, Berta Cillero-Pastor, Emeline M. Van Craenenbroeck and Pieter-Jan Guns (UAntwerp)

11.30-11.45 **Identification of the Endogenous Co-agonist of NMDA Receptor in Dopamine Neurons of the Substantia Nigra**
Sofian Ringlet, Vincent Seutin, Dominique Engel (ULiège)

11.45-12.00 **Hepatic lipid flux damages the liver during starvation-induced steatosis**
Macarena Pozo-Morales, Ansa Cobham, Cielo Centola, Nicolas Rohner and Sumeet Pal Singh. (ULB & Stowers Institute for Medical Research, Kansas City, USA)

12.00 – 12.30 Lunch

12.30 – 13.30 Guided Poster Session

Posters:

- **Study of the reversibility of the membrane hyperpolarization during mammalian sperm capacitation**
  Bertrand de Prelle, David Pening, David Gall, Pascale Lybaert (ULB)
- **Kino2omics Commitment to excellence in biomarker and target discovery**
  Claudina Perez Novo, Steven Van Laere, Wim Vanden Berghe (UAntwerp)
- **A non-invasive partial ablation study in zebrafish liver**
  Sema Elif Eski, Macarena Pozo-Morales, Sumeet Pal Singh (ULB)
- **FoxO1, Do we know it all?**
  Inés Garteizgogeascoa Suñer, Alejandra Vargas Valderrama, Liu Peiduo, Bai Hua, Frederic Lemaigre, Sumeet Pal Singh (ULB)
- **Gasdermin E-mediated secondary necrosis in atherosclerosis**
  Elena Beltran Salgado, Michelle Zurek, Guido De Meyer, Wim Martinet (UAntwerp)
- **The Role of Elastin-Derived Peptides on Vascular Smooth Muscle Cell Function**
  Hamutcu A., Van Praet M., De Meyer G.R.Y., Martinet W., Roth L. (UAntwerp)

Oral communications (afternoon session)

13.30-13.45 **Sirtuin 6 protects vascular smooth muscle cells from senescence and reduces atherosclerosis**
M.O.J. Grootaert, A.K. Uryga, A. Finigan, N.L. Figg, M.R. Bennett (University of Cambridge, UK & KULeuven)

13.45-14.00 **ERBB4-Selective and Sustained activation by NRG1 attenuates atrial fibrosis and fibrillation**
J. Van fraeyenhove, M. Tubeeckx, E. Feyen, T. Bruyns, G.R. Y. De Meyer, S. Murphy, H. Heidbuchel, V.F.M Segers, G.W. De Keulenaer (UAntwerp)

14.00-14.15 **Finding new targets to modulate food intake**
Dayana Abboud, Julien Hanson (ULiège)

Closing lecture

14.15-15.00 **Calcium microdomains in T cells through the lens of computational modelling**
Geneviève Dupont (ULB)

15.00 Coffee – Tea and Networking

15.30 General Assembly for the Members

ORAL PRESENTATION 1

High Pulsatile Load Reversibly Inhibits Aortic FAK Activity And Decreases Viscoelasticity

Cédric H.G. Neutel, Callan D. Wesley, Guido R.Y. De Meyer, Wim Martinet, Pieter-Jan Guns

Laboratory of Physiopharmacology, University of Antwerp, Belgium

INTRODUCTION | The aortic wall is composed of different functional elements, such as extracellular matrix proteins and vascular smooth muscle cells (VSMCs) that together define its viscoelastic properties. Focal adhesion (FA) proteins connect the extracellular matrix to the actin cytoskeleton of the cell. FA transmits mechanical forces to and from the cell and act as a biochemical signalling hub.

METHODS | Aortic segments from C57Bl6/J mice were mounted in a Rodent Oscillatory Set-up for Arterial Compliance (ROTSAC) and subjected to high frequency cyclic stretch. Diastolic and systolic diameter were determined. Viscous modulus (Eη) was extracted from pressure-diameter tracings by eliminating loop hysteresis. Afterwards, the elastic modulus (EE) was calculated as the slope of the resulting pressure-diameter tracing. Phenylephrine (2 µM, PE) was used to elicit VSMC contraction and focal adhesion kinase (FAK) activation. FAK activity was analyzed by measuring the amount of phosphorylated FAK via western blotting.

RESULTS | PE increased both Eη and EE and increased FAK activity. Moreover, increasing pulsatile load, by increasing pulse pressure from 40 to 90 mmHg, decreased both PE-induced aortic viscoelasticity and PE-induced FAK activity. This effect was reversible, since 15 minutes after the “pulsatile bout”, viscoelastic parameters and FAK activity were restored.

CONCLUSION | High pulsatile load is able to decrease PE-induced viscoelastic changes and represses FAK activity.

ORAL PRESENTATION 2

Arterial Stiffness Is Transient During Doxorubicin Treatment Despite Persistent Endothelial Cell Dysfunction: a Longitudinal Study Complemented by Proteomics

Matthias Bosman, Dustin N. Krüger, Charles H. Van Assche, Cédric H.G. Neutel, Kasper Favere, Constantijn Franssen, Wim Martinet, Lynn Roth, Guido R.Y. De Meyer, Berta Cillero-Pastor, Emeline M. Van Craenenbroeck and Pieter-Jan Guns

University of Antwerp, Laboratory of Physiopharmacology

INTRODUCTION | The chemotherapeutic doxorubicin (DOX) induces vascular toxicity, which is clinically relevant in cancer survivors since arterial stiffness and endothelial cell (EC) dysfunction are strong, independent markers for future cardiovascular risk. However, clinical studies have reported conflicting results. Accordingly, we aimed to longitudinally evaluate vascular function in a DOX-treated murine model complemented by a mechanistic proteomics approach.

METHODS | Mice were injected intraperitoneally with vehicle, low (2 mg/kg) or high DOX dose (4 mg/kg) once per week for 6 weeks with follow-up until 15 weeks. Arterial stiffness was assessed longitudinally in vivo and ex vivo complemented by vascular reactivity evaluation. Proteomics was performed with functional enrichment analysis using Enrichr (Reactome) to obtain molecular insight.

RESULTS | Treatment with DOX revealed a biphasic response in arterial stiffness, characterised by an initial increase and a subsequent decrease. However, EC dysfunction persisted during treatment, evidenced by impaired endothelium-dependent acetylcholine-induced relaxation, decreased basal nitric oxide index and reduced phosphorylation of Ser1177-endothelial nitric oxide synthase. After treatment (follow-up), both arterial stiffness and EC dysfunction recovered. Furthermore, proteomics and enrichment analysis revealed pathways associated with EC injury response and extracellular matrix (ECM) remodelling. The upregulated proteins vitronectin, thrombospondin-1 and collagen XIV, which were also validated with immunohistochemistry, proved to mediate DOX-induced ECM remodelling, and possibly drive the decrease in arterial stiffness.

CONCLUSION | DOX induces a transient increase in arterial stiffness despite persistent EC dysfunction, placing arterial stiffness as a time-sensitive and variable parameter within DOX-induced cardiovascular toxicity.

ORAL PRESENTATION 3

Identification of the Endogenous Co-agonist of NMDA Receptor in Dopamine Neurons of the Substantia Nigra

Sofian Ringlet, Vincent Seutin, Dominique Engel

GIGA Neurosciences université de Liège

Introduction | Dopamine (DA) neurons in the substantia nigra are vital for the control of movement. This fundamental function is encoded by the output signal of DA neurons which switches from spontaneous firing to bursts of action potentials (APs). The latter produce an increase of dopamine release in targeted areas which is associated to locomotion initiation. The activation of the N-methyl-D-aspartate receptors (NMDARs), by the simultaneous binding of glutamate and a co-agonist, is indispensable for the generation of this physiological signal. While both D-serine and glycine can potentially serve as endogenous agonist at the NMDAR glycine site, its identity is correlated to development, synapse specificity, NMDAR subunits and level of synaptic activity. However, in nigral DA neurons the identity of the endogenous co-agonist remains unknown.

Methods | Identification of the main co-agonist is determined by using glycine and D-serine specific degradation enzymes during whole-cell patch-clamp recordings of NMDA-mediated synaptic transmission, extrasynaptic tonic current and on pharmacologically-induced bursts in brain slices from wild-type Wistar rats (4-6 weeks old).

Results | D-serine degradation significantly reduced spontaneous excitatory transmission while glycine depletion significantly reduced NMDA-mediated tonic current. D-serine degradation did not alter the frequency, number of AP per bursts. In contrast, glycine removal did not change the number of AP per bursts but significantly decreased the frequency of the bursts.

Conclusion | D-serine is the endogenous co-agonist of synaptic NMDARs while glycine is the main one for extrasynaptic NMDARs. Regarding the bursting activity, glycine seems to be the co-agonist sustaining such firing pattern.

ORAL PRESENTATION 4

Hepatic lipid flux damages the liver during starvation-induced steatosis

Macarena Pozo-Morales (1), Ansa Cobham (2), Cielo Centola (2), Nicolas Rohner (2) and Sumeet Pal Singh (1)

(1) Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (I.R.I.B.H.M.), Université Libre de Bruxelles, Brussels, Belgium. (2) Stowers Institute for Medical Research, Kansas City, MO, USA

INTRODUCTION | During starvation, vertebrates utilize the liver as lipid storage organ. For instance, patients suffering from anorexia nervosa, overnight fasted mice and starved zebrafish larvae develop fatty liver. Like non-alcoholic fatty liver diseases, starvation-induced steatosis leads to liver damage. However, the role of steatosis in liver damage is unclear. Particularly, it is unknown if resolution of steatosis is beneficial for the liver and survival.

METHODS | To evaluate steatosis in fish, animals were stained with Nile Red to label lipid droplets. We previously demonstrated that hepatic steatosis is regulated by calcium signaling in zebrafish liver. To resolve hepatic steatosis during starvation, we enhanced intracellular calcium flux by mobilization of calcium stores from lysosomes. For this, we utilized an agonist for TPC2, a lysosomal cation channel. Animals were accessed for lifespan curve, liver inflammation, atrophy.

RESULTS | We show that starvation leads to an infiltration of macrophages in the liver that phagocytose hepatocytes. Further, mobilization of endo-lysosomal calcium stores efficiently reduces lipid droplets in the liver. However, the resolution of steatosis enhanced macrophage infiltration, hepatocyte phagocytosis and starvation-induced mortality. To understand how animals with enhanced starvation resistance deal with hepatic steatosis and liver damage, we investigated Mexican cavefish and found, surprisingly, that cavefish do not accumulate lipid droplets in the liver upon starvation and are protected from liver damage.

CONCLUSION | Our study demonstrates that the turnover of lipid droplets in hepatocytes during starvation is detrimental to liver health and identifies a potential protective mechanism in cavefish against liver atrophy.

ORAL PRESENTATION 5

Sirtuin 6 protects vascular smooth muscle cells from senescence and reduces atherosclerosis

M.O.J. Grootaert, A.K. Uryga, A. Finigan, N.L. Figg, M.R. Bennett

University of Cambridge – KULeuven

INTRODUCTION | Vascular smooth muscle cell (VSMC) senescence promotes atherosclerosis, partly due to lipid-mediated DNA damage and telomere dysfunction. Sirtuin 6 (SIRT6) deacetylase is involved in DNA damage signaling, inflammation and metabolism. We examined SIRT6 expression in human VSMCs (hVSMCs), the role, regulation and downstream pathways activated by SIRT6, and how VSMC SIRT6 regulates atherogenesis.

METHODS | Lentiviruses were used to stably knockdown SIRT6 using shRNA, or overexpress SIRT6 or its deacetylase-inactive mutant (SIRT6H133Y) in human VSMCs. To study its role in atherosclerosis, ApoE$^{-/-}$ mice were generated that overexpress SIRT6 or SIRT6H133Y in VSMCs only.

RESULTS | SIRT6 protein, but not mRNA, expression was reduced in VSMCs in human and mouse atherosclerosis, and in hVSMCs derived from plaques or undergoing replicative or palmitate-induced senescence vs. healthy aortic VSMCs. SIRT6 bound to telomeres, while SIRT6 inhibition using shRNA or expression of a deacetylase-inactive mutant (SIRT6H133Y) shortened hVSMC lifespan and induced senescence, associated with telomeric H3K9 hyperacetylation and 53BP1 binding, indicative of telomere damage. SIRT6 overexpression however preserved telomere integrity, delayed cellular senescence, and prevented senescence-associated changes in metabolism and inflammatory cytokine expression. In vivo, SM22α-hSIRT6/ApoE$^{-/-}$ mice showed reduced atherosclerosis, plaque senescence and inflammation compared to controls, while plaques of SM22α-hSIRT6H133Y/ApoE$^{-/-}$ mice showed increased features of plaque instability.

CONCLUSION | SIRT6 protein expression is decreased in human and mouse plaque VSMCs. SIRT6 regulates telomere maintenance and VSMC lifespan, and inhibits atherogenesis, all dependent on its deacetylase activity. Hence, endogenous SIRT6 is an important inhibitor of VSMC senescence and atherosclerosis.

ORAL PRESENTATION 6

ERBB4-Selective and Sustained activation by NRG1 attenuates atrial fibrosis and fibrillation

J. Van fraeyenhove, M. Tubeeckx, E. Feyen, T. Bruyns, G.R. Y. De Meyer, S. Murphy, H. Heidbuchel, V.F.M Segers, G.W. De Keulenaer

University of Antwerp

Introduction | Atrial fibrillation (AF) results from electrical and structural remodeling of the atria, in which inflammation and fibrosis play a role. Current therapy is limited to antiarrhythmic drugs and ablations, but does not target the structural problem. Recent studies showed that neuregulin-1 (NRG1), an epidermal growth factor family member, has anti-fibrotic and anti-inflammatory effects in the myocardium.
Purpose | To test the effects of JK07, a NRG1 antibody fusion comprising an ERBB3 antagonistic antibody which selectively signals through ERBB4 preferentially over ERBB3, on atrial fibrosis and AF inducibility.
Methods | Atrial samples were harvested from male rats (Wistar Han, 10 weeks old), cut into small pieces (1-2mm2) and kept in low serum medium in the presence or absence of JK07 (5nM). Col1a1 and Col3a1 mRNA was quantified after 24-72 hours. AF inducibility was tested in a first AF model in which male mice (C57BL/6N, 12-15 weeks old) were treated with angiotensin-II (Ang-II, 4 weeks, osmotic mini-pumps, 3000 ng/kg/min), and in a second AF model in which mice were fed with a high fat diet (HFD, 8 weeks, 60% Kcal fat) inducing severe weight gain (56±3% increase compared to 23±4% with regular chow). In both models, AF inducibility was tested by 5 runs of programmed electrical stimulation (PES) with a trans-jugular octapolar catheter. AF inducibility (% mice inducible by ≥3 PES-runs) and duration of PES-induced AF (AF duration) were recorded. Mice were randomized for treatment with vehicle or JK07 (2x/week, 1mg/kg, IV, n=5-7/group).
Results | In cultured atrial samples, Col1a1 and Col3a1 mRNA expression gradually increased up to 2-3 fold over 3 days. JK07 robustly attenuated this effect by 59±17% ($p<0.05$). In mice, both Ang-II and HFD significantly increased AF inducibility and AF duration. In Ang-II mice, JK07 attenuated AF inducibility (from 57% to 20%) and AF duration (from 33.3 ± 15.1 to 1.5 ± 1s). In HFD mice, JK07 significantly attenuated AF inducibility (from 57% to 0%) and AF duration (from 10.9±3.2s to 0.76±0.5s, $p<0.05$).
Conclusion | These results show anti-fibrotic effects by selective ERBB4 stimulation with JK07 in atrial tissue in vitro, together with AF-preventive effects in two unrelated mouse models.

ORAL PRESENTATION 7

Finding new targets to modulate food intake

Dayana Abboud [1], Julien Hanson [1,2]

(1) Laboratory of Molecular Pharmacology, GIGA-Molecular Biology of Diseases, University of Liège, Liège, Belgium. (2) Laboratory of Medicinal Chemistry, Centre for Interdisciplinary Research on Medicines (CIRM), University of Liège, Belgium.

Obesity is a major burden to public health as it increases the risk of several chronic diseases, like diabetes, cardiovascular diseases and cancer. Energy homeostasis is centrally regulated by the hypothalamus where the arcuate nucleus (ARC) orchestrates feeding behaviors and energy balance. Two neuronal populations of the ARC are considered as the first order neurons mediating the effects of various signals such as leptin and insulin, the orexigenic NPY/AgRP neurons and the anorexigenic POMC/CART neurons. Leptin is an adipocyte-secreted peptidic hormone that inhibits food intake and regulates body weight. Nearly in all forms of obesity, circulating levels of leptin increase but fail to suppress food intake and body weight. This condition has been identified as "leptin resistance". G protein-coupled receptors (GPCRs) are the largest family of membrane proteins and a successful source of drug targets. However, many GPCRs are understudied or even orphans meaning they have no known ligands. Despite our knowledge of the circuits regulating feeding, we have been unable to deliver effective drugs targeting these systems. The failure in targeting the melanocortin pathways comes out partly from an incomplete understanding of leptin resistance.

Here, we propose to investigate the interplay between the signaling of orphan GPCR and leptin receptor in leptin-sensitive cells of the ARC. To do so, we will use conditional knockout animals and pharmacological modulators. We will measure various metabolic parameters including weight and food intake. We will also establish hypothalamic cell cultures in which we will address the impact of G protein signaling on leptin.

POSTER PRESENTATION 1

Study of the reversibility of the membrane hyperpolarization during mammalian sperm capacitation

Bertrand de Prelle, David Pening, David Gall, Pascale Lybaert

Research Laboratory on Human Reproduction, Université Libre de Bruxelles

INTRODUCTION | When mammalian spermatozoa are released in the female reproductive tract, they are incapable of fertilizing the oocyte. They need a prolonged exposure to the alkaline medium of the female genital tract before their flagellum gets hyperactivated and the acrosome reaction can take place, allowing the sperm to fuse with the oocyte. Even though capacitation has been shown to be reversible, it has never been shown that hyperpolarization can be reversed back in uncapacitating medium. We present here the results of our experiments aimed at validating the reversibility of hyperpolarization that is predicted by our mathematical model.

METHODS |F1 mouse sperm populations were collected from epididymides and incubated for one hour into capacitating medium. The samples were then centrifugated and resuspended in uncapacitating conditions. Vm measurements was performed using population fluorimetry with the positively charged carbocyanine probe DiSC3(5). After the initial fluorescence stabilization, the calibration was performed by adding valinomycin and sequential additions of KCl, providing the conversion of fluorescence levels into membrane potentials.

RESULTS | Preliminary results validate the reversibility of the bicarbonate induced hyperpolarization in murine sperm populations. This reversibility seems partial in the sense that the Vm does not reach back the initial membrane potential.

CONCLUSIONS | This is the first time that the reversibility of the bicarbonate induced hyperpolarization can be observed in mammal sperm. Even though our experiment's statistics shall be increased, the incompleteness of reversibility might be due to an insufficient decapacitation duration; that will be tested in the future.

POSTER PRESENTATION 2

Kino2omics Commitment to excellence in biomarker and target discovery

Claudina Perez Novo, Steven Van Laere, Wim Vanden Berghe

University of Antwerp

Protein phosphorylation is a reversible post-translational modification and a crucial mechanism for regulating cellular functions, such as proliferation, cell cycle, apoptosis, motility, growth, and differentiation. Protein kinases and phosphatase control this process. Deregulated kinases are frequently found to be oncogenic and can be central to the survival and spread of cancer cells. The PPES service facility for Global Kinase Activity profiling: Kino2Omics performs parallel measurement(s) of kinase activities by recording phosphorylation changes in real-time, revealing a more realistic view of the cellular signalling states and molecular mechanisms operating in disease and drug mode of action. The facility uses the Pamstation-12, a fully automated instrument designed to process peptide microarrays (pamchips). This technology allows simultaneous measurement of multiple kinase activities (either activation or inhibition) through highly sensitive monitoring of the phosphorylation dynamics of 144 peptide substrates by Serine/ Threonine or 196 for Tyrosine kinases, present in (clinical) biosamples of interest (cells, tissues, organoids, ipscs, patients biopts). Moreover, the platform allows the “pharmacology on-chip” evaluation of kinase inhibitors that, together with the new systems biology core that integrates kinome data with other omics techniques (i.e., transcriptome, phospho-proteome, and epigenome data), will narrow the bridge between disease and drug discovery strategies. Identifying essential pathology-related kinases to identify viable targets in patients has led to a notable improvement in clinical disease management and constitutes an important step as we progress into the age of personalised medicine.

POSTER PRESENTATION 3

A non-invasive partial ablation study in zebrafish liver

Sema Elif Eski, Macarena Pozo-Morales, Sumeet Pal Singh

ULB

INTRODUCTION | The liver harbors an incredible capacity to recover from injury since it serves as the body's main line of defense against endogenous and external stresses. However, there is still controversy about the relative contribution of different cellular sources towards hepatocyte regeneration, and thereby to liver recovery. Current injury models dictate invasive methods for liver injury and the extent of the injury can vary the regenerative responses. Here, we aim to investigate the sources of zebrafish liver regeneration using a non-invasive partial ablation model and further compare the distinguishing characteristics of contributing cells during liver regeneration.

METHODS | I developed a combinatorial Cre-Lox system where the default blue color is changed by inducible Cre activity to either mCherry-nitroreductase (NTR) or EGFP in the entire hepatocyte population. NTR-expressing hepatocytes are specifically killed, while sparing EGFP-expressing hepatocytes, using Mtz administration. The contribution and characteristics of spared hepatocytes, in green, vs de novo formed hepatocytes, in blue, are accessed during regeneration.

RESULTS | Upon partial ablation, de novo hepatocytes contributed robustly to liver regeneration even in the presence of spared hepatocytes. At early stages of regeneration, the spared and de novo hepatocytes differed in their lipid content, accessed by Nile Red staining.

CONCLUSION | Here we demonstrate a non-invasive partial ablation system for zebrafish liver that enables comparative analysis of the regenerative lineages. We show that in zebrafish, de novo generation of hepatocytes does not require complete ablation. Further, newly generated hepatocytes display a transient incapacity to collect lipids, suggesting immature characteristics.

POSTER PRESENTATION 4

FoxO1, Do we know it all?

Inés Garteizgogeascoa Suñer, Alejandra Vargas Valderrama, Liu Peiduo, Bai Hua, Frederic Lemaigre, Sumeet Pal Singh.

IRIBHM-ULB

INTRODUCTION | Adaptation to changes in nutrient availability is critical for all organisms. Fasting is present in pathological situations such as eating disorders but also in different religious and cultural practices. The liver represents a metabolic hub allowing vertebrates to cope with these nutritional challenges. At the hepatocyte level, nutritional inputs induce a complex array of changes in posttranslational modifications (PTMs) controlling protein function.

METHODS | In this study, we aim to investigate the effects of a fasting-refeeding regime, a model of irregular eating, on the hepatic foxo1a PTMs using zebrafish as a model organism.

RESULTS | FoXO1 is a key regulator of hepatic glucose production and lipid metabolism. Its activity is regulated by phosphorylation affecting its subcellular distribution, DNA binding, and degradation. We identified that contrary to our current knowledge starvation reduces the amount of FoxO1 in the nucleus whereas activation of insulin signaling during (re)feeding is unable to promote nuclear exclusion of FoxO1. Moreover, we also observe the latte in mouse embryonic liver and in Drosophila.

CONCLUSION | We will use zebrafish to study the dynamics, regulation, and function of FoxO1, a conserved transcription factor under both low and high nutrient conditions in this new biological paradigm.

POSTER PRESENTATION 5

Gasdermin E-mediated secondary necrosis in atherosclerosis

Elena Beltran Salgado, Michelle Zurek, Guido De Meyer, Wim Martinet

Laboratory of Physiopharmacology, Infla-Med Research Consortium of Excellence, University of Antwerp

INTRODUCTION | Atherosclerosis is a progressing disease characterized by inflammation and lipid deposition inside the arterial wall, leading to plaque formation and possible cardiovascular complications. Necrotic cell death stimulates atherogenesis through the induction of inflammation and enlargement of the necrotic core region of atherosclerotic plaques. Plaques with a large necrotic core are susceptible to rupture, the necrotic core is not only critical for plaque stability but also for thrombogenicity. GSDME perforates cellular membranes during apoptosis-driven secondary necrosis. This type of cell death is considered the most common cause of necrosis in atherosclerotic plaques and results from the lack of efficient phagocytic clearance (efferocytosis) of apoptotic cells. In addition, GSDME is transcriptionally controlled by p53 and essential in a p53-mediated response to DNA damage. Although caspase-3-dependent apoptosis followed by secondary necrosis is an important feature of atherosclerotic plaque development, it is surprising that the role and impact of GSDME-mediated secondary necrosis in atherosclerosis is not yet studied.

AIM | Inducing DNA damage in macrophages to stimulate apoptosis and GSMDE expression to confirm secondary necrosis.

METHODS | J774 macrophages are treated with doxorubicin, etoposide, and tert-butylhydrogenperoxide in different concentrations and treatment periods. Neutral red assay is used as an indication of cell viability. The expression of total and N-terminal cleaved GSDME, total and active p53, and cleaved caspase-3 in treated J774 cells and mouse plaques are analyzed via western blotting. Comet assay will be used to indicate DNA damage, TUNEL assay will confirm apoptosis and PI staining will measure necrosis.

POSTER PRESENTATION 6

The Role of Elastin-Derived Peptides on Vascular Smooth Muscle Cell Function

Hamutcu A., Van Praet M., De Meyer G.R.Y., Martinet W., Roth L.

Laboratory of Physiopharmacology, University of Antwerp

Introduction | Elastin is responsible for the elasticity of the vessel wall, but due to repetitive stretches and relaxations as we age, it will fracture, leading to arterial stiffness and the release of soluble elastin derived peptides (EDPs). Since arterial stiffness is an important driving force of cardiovascular disease, we aim to investigate whether EDP-related signaling can contribute to this process by affecting vascular smooth muscle cell (VSMC) migration, collagen production and autophagy.

Methods | L929 fibroblasts and primary mouse VSMCs were treated with kappa-elastin (kE, 50□g/mL) for 30min and 4h in the presence or absence of bafilomycin (160nM). Cell lysates were analyzed by western blotting to determine autophagy levels (LC3, p62) and involved signaling pathways (AKT, ERK or AMPK). To measure collagen production, mouse VSMCs were treated with kE (50□g/mL, 48h). Collagen was stained with Sirius red and quantified by measuring absorbance. For cell migration analysis, L929 fibroblasts were treated with kE (50□g/mL, 18h) and a wound healing assay was performed.

Preliminary results | Treatment of VSMCs with kE for 30min activated AKT and ERK signaling whereas AMPK seemed downregulated. After 4h, these effects could not be observed, but we did detect a slight decrease in autophagy flux. Upon treatment with kE, migration appeared lower than in control conditions, but no effect on collagen production was observed.

Conclusion | These preliminary data in VSMCs and fibroblasts show a slight inhibition in autophagy flux by EDPs, which can be related to the activation of AKT and ERK and inhibition of AMPK. Collagen production was not changed, but migration was slightly decreased. However, more research is required to fully understand the potential role of EDPs in arterial stiffness development.