EUROPEAN SOCIETY FOR MICROCIRCULATION

BIENNIAL MEETING

ESM 2025

MAY 19-22 • 2025 • SZEGED • HUNGARY

PROGRAMME

EUROPEAN SOCIETY FOR MICROCIRCULATION
Integrating
Vascular Biology & Medicine

Dear Colleagues,

On behalf of the European Society for Microcirculation (ESM), the Local Organizing Committee and Program Committee, we wish you a warm welcome to the Biennial Meeting of the European Society for Microcirculation (ESM 2025), to be held in Szeged, Hungary between May 19-22, 2025. The venue of the meeting will be the József Attila Study and Information Centre of the University of Szeged.

The conference is a regular international meeting of the ESM scientific society, which has been held more than 30 times since 1959 in various European cities. On this occasion, the Hungarian Society for Microcirculation and Vascular Biology (MMVBT) is proud to host ESM 2025! In addition, the organization of the conference will be shared with the Hungarian, Croatian and Slovenian Physiological Societies to showcase research in microvascular physiology in the region.

The ESM and MMVBT aim to create a common platform for clinical and basic research focusing on the microcirculation of organs and tissues. From a medical perspective, the conference theme is of central importance, as microvascular pathologies contribute significantly to many chronic diseases such as cardiovascular and central nervous system disorders.

The ESM 2025 conference will provide an excellent opportunity for new international collaborations and active integration into the international vascular research community. ESM 2025 is an event that provides a platform for innovation in international partnership, especially for the development of therapeutic procedures and technologies that can be used in medicine.

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GENERAL INFORMATION

Onsite Registration

SZTE József Attila Study and Information Centre, Szeged, Hungary

May 19, 2025 from 12 am

The organisers kindly ask participants to please come to the registration desk first. They are also kindly requested to wear their name badges until the end of the Meeting.

Presentations

SZTE József Attila Study and Information Centre, Szeged, Hungary – Congress Hall, Lecture Room I, Lecture Room II

Participants wishing to present a lecture are requested to prepare their presentation in MS PowerPoint format in English and hand it to the technician in the auditorium at the latest during the last coffee break before the presentation. Presentations cannot be modified at this point. The presentations will be copied onto the computer by the technician colleague who will be available to the presenters during their presentation. The language of the presentation is English. Technicians will provide a control monitor, a wireless presenter including a laser pointer and a countdown timer monitor each room.

Poster session

SZTE József Attila Study and Information Centre, Szeged, Hungary – Social area

Posters should be exhibited upon arrival and kept outside until 12:30 pm on Thursday May 22, the end of the Conference. Organisers will provide poster stands and all necessary equipment to hang the posters on it. Poster size: 90 cm (height) 150 cm (width), horizontal format.

The guided poster session will take place on Wednesday, May 21, 2025 from 16:20 to 18:00 and Poster presenters are kindly requested to be present in person during the Poster Session to answer questions from interested parties.

The Poster Award ceremony will take place at the closing of the Meeting. May 22. Five such awards (100 Euro each), will be given for the best posters.

Industrial exhibition, coffee break, lunches

Social area, SZTE József Attila Study and Information Centre, Szeged, Hungary

Social programme

Monday, May 19, 2025, 18:00, Atrium, SZTE József Attila Study and Information Centre, Szeged, Hungary: **Welcome reception**

Wednesday, May 21, 2025, 19:00, Fehértói Fisherman's Lodge, Szeged, Hungary: **Networking dinner (Gala dinner)**

Arrival, departure

To *check in* at the hotels, you must present your ID. This rule applies to all accommodation in Hungary. Please make sure you are aware about your accommodation *check in* and *check out* time. Check-out after the official check out time will incur an additional charge.

Program code explanation

First letter of the name of days: M- Monday, T-Tuesday, W-Wednesday, T-Thursday

Second letter is the venue of the lectures: **C**- Congress Hall, **LI**- Lecture Room I, **LII**- Lecture Room II

Numbers:

1st number: number of the section within a day

2nd number: serial number within a section

The number before the title of the abstract explains the number in JVR.

Exhibitor game for the participants on ESM 2025

Dear Participant,

High-value gift packages wait for their owners, which will be raffled at the closing of the Meeting on May 22, among the participants who participate in the announced prize draw. The prize draw takes place as follows:

When visiting the exhibition, you need to collect stamps/signature from the representative of each company at each stand. Anyone who obtains the necessary stamp/signature at all stands and, based on the information gathered during the stand visit, correctly answers the questions concerning the exhibitors will participate in the draw. Printed forms are available at the exhibition area and at the registration desk. Completed forms needs to be handle to the registration staff by 10:00 am on Thursday at the registration desk.

Those who participate in the draw in person are entitled to the prize.

Thank you for your participation and we wish you a meaningful time!

The organizers

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May 19, 2025 Monday

12:00-13:30		**ESM Board Meeting**
14:00-17:00	**M-C1**	**Pre-conference course 180'** Chair: Ferenc Bari
14:00-14:30	**M-C1-1**	**Martin Leahy 25+5'** University of Galway, Ireland *1-Fifty years of microcirculation monitoring and imaging technology*
14:30-15:00	**M-C1-2**	**Pia Pelaez 25+5'** Iconeus, France *2-Symposium by Iconeus: From brain function to microvessels: Functional ultrasound and super-resolution vascular imaging with Iconeus One*
15:00-15:30	**M-C1-3**	**Zsolt Piroth 25+5'** Gottsegen National Cardiovascular Center, Hungary *3-Microvascular angina: significance, evaluation and treatment of coronary microvascular dysfunction*
15:30-16:00		**Coffee break and industrial exhibition**
16:00-16:30	**M-C1-4**	**Hamid Dehghani** University of Birmingham, United Kingdom *4-Applications of diffuse optics for detection and characterisation of disease*
16:30-17:00	**M-C1-5**	**Fan Yu 30'** Animalab Hungary Ltd./ RWD Life Science Co., LTD *5-Symposium by Animalab Hungary Ltd./ RWD Life Science Co., LTD: Laser Speckle Contrast Imaging (LSCI): A Transformative Tool for Decoding Microvascular Dynamics in Health and Disease*
17:00-17:30		**Welcome and opening**
17:30-18:10	**M-C2**	**OPENING LECTURE – David Attwell 40'** University College London, United Kingdom *6-Regulation of blood flow at the capillary level in health and disease* Chair: Eszter Farkas
18:10-19:30		**Reception**

May 20, 2025 Tuesday

8:30-9:20 **T-C1** **KEYNOTE LECTURE – Edina Cenko 50'**
University of Bologna, Italy
7-Mechanistic insights into coronary microvascular disease
Chair: Ines Drenjančević

9:30-10:50 **T-C2** **Coronary endothelial dysfunction 80'**
Chairs: Paul A. Fraser, Amanda J. Leblanc

T-C2-1 **Paul A. Fraser 20+5'**
King's College London, London, United Kingdom
8-Endothelial derived hyperpolarization has a role coronary microvascular dysfunction

T-C2-2 **Amanda J LeBlanc**[1], Brad Hill[1], Andreas Beyer[2], William Hughes[2], Cristhian Gutierrez[2] **20+5'**
[1]Center for Cardiometabolic Sciences, University of Louisville, United States
[2]The Medical College of Wisconsin, United States
11-Mitochondrial ROS Influence effect on microvascular ageing

T-C2-3 **Bence Varga**[1], Mária Szekeres[1,2], Ágnes Cser[3], Gabriella Dörnyei[2], György L. Nádasy[1], Zoltán Benyó[3], Akos Koller[2, 3, 4] **10+5'**
[1]Department of Physiology, Semmelweis University, Budapest, Hungary
[2]Department of Morphology and Physiology, Semmelweis University, Budapest, Hungary
[3]Institute of Translational Medicine, Semmelweis University, Budapest, Hungary
[4]Department of Physiology, New York Medical College, Valhalla, NY, United States
155- Differences in vasomotor responses of extra- and intracranial arteries isolated from young and aged pregnant rats

T-C2-4 **Imre Babay**[1], Nóra Melinda Kerkovits[1], Mónika Kosztelnik[1,2], Csillag Virág Tóth[1], Balázs Besztercei[1], Anna Janovicz[1,2], Aliz Majer[1], Krisztina Vén[1,4], Éva Ruisanchez[1,2], Gábor Szénási[1], Tamás Radovits[3], Ágnes Szappanos[3,5], József Balla[6], Gábor Csányi[7,8], Béla Merkely[3], Péter Sótonyi[3], Zoltán Benyó[1,2] **10+5'**
[1]Institute of Translational Medicine, Semmelweis University, Budapest, Hungary
[2] HUN-REN-SU Cerebrovascular and Neurocognitive Disease Research Group, Budapest, Hungary
[3]Heart and Vascular Center, Semmelweis University, Budapest, Hungary
[4]Department of Neurology, Semmelweis University, Budapest, Hungary

[5]Department of Rheumatology and Clinical Immunology, Semmelweis University, Budapest, Hungary
[6] Institute of Internal Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
[7]Vascular Biology Center, Medical College of Georgia, Augusta University, Augusta, GA, United States
[8]Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta University, Augusta, GA, United States
164- C3a anaphylatoxin signaling: Bidirectional vasoactive effects in health and vascular disease

9:30-10:50 **T-LI1** **Microvascular research in Hungary presented by the Hungarian Physiological Society 80'**
Chairs: Zoltán Benyó, Ferenc Bari

T-LI1-1 **Zoltán Jakus 15+5**
Department of Physiology, Semmelweis University, Budapest, Hungary
12-Nucleoside-modified mRNA mediated modulation of organ-specific lymphatic growth and function in preclinical animal models

T-LI1-2 **Ágnes Kemény[1,2]**, Szabina Horváth[3], Areej Jaber[3], Erika Pintér[2], Rolland Gyulai[3,4] **15+5**
[1]Department of Physiology and Biochemistry, University of Veterinary Medicine, Budapest, Hungary
[2]Department of Pharmacology and Pharmacotherapy, University of Pécs, Medical School, Pécs, Hungary
[3]Department of Dermatology, Venereology and Oncodermatology, University of Pécs, Medical School, Clinical Centre, Pécs, Hungary
[4]Department of Dermatology and Allergology, Albert Szent-Györgyi Health Center, Szeged, Hungary
13-Development and evaluation of a long-term imiquimod-induced psoriasiform dermatitis model in Balb/c and C57BL/6J mice

T-LI1-3 **Szabolcs Péter Tallósy,** László Juhász, Roland Fejes, Attila Rutai, József Kaszaki, Mihály Boros, Dániel Érces, Gabriella Varga, Marietta Poles, Andrea Szabó **15+5**
Institute of Surgical Research, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary
14- Microcirculatory impairment as a precursor to mitochondrial dysfunction in experimental sepsis

T-LI1-4 **László Hricisák[1,2],** Éva Pál[1,2], Dorina Nagy[1,2,3,4,5], Andreas Polycarpou[1,6,7], Ágnes Fülöp[1,2], András Iring[1,8], Éva Ruisanchez[1,2], Péter Sándor[1,2], Zoltán Ungvár[3,4,5,9,10], Péter Sótonyi[11], Zoltán Benyó[1,2] **15+5'**

[1]Institute of Translational Medicine, Semmelweis University, 1094 Budapest, Hungary
[2]HUN-REN-SU Cerebrovascular and Neurocognitive Diseases Research Group, Budapest, Hungary
[3]Vascular Cognitive Impairment, Neurodegeneration and Healthy Brain Aging Program, Department of Neurosurgery, University of Oklahoma Health Sciences Center, Oklahoma City, United States
[4]Oklahoma Center for Geroscience and Healthy Brain Aging, University of Oklahoma Health Sciences Center, Oklahoma City, United States
[5]International Training Program in Geroscience, Doctoral College/Institute of Preventive Medicine and Public Health, Semmelweis University, Budapest, Hungary
[6]Mayo Clinic, Department of Surgery, Rochester, United States
[7]Division of Cardiothoracic Surgery, University of Minnesota, Minneapolis, United States
[8]Laboratory of Molecular Pharmacology, HUN-REN Institute of Experimental Medicine, Budapest, Budapest, Hungary
[9]Stephenson Cancer Center, University of Oklahoma, Oklahoma City, United States
[10]Department of Health Promotion Sciences, College of Public Health, University of Oklahoma Health Sciences Center, Oklahoma City, United States
[11]Department of Vascular and Endovascular Surgery, Semmelweis University, Budapest, Hungary
15-Spatiotemporal adaptation of the cerebrocortical microcirculation to unilateral carotid artery occlusion: the role of NO

9:30-10:50 **T-LII1** **Vasodilation with clinical relevance – large animal models and mechanistic insights 80’**

Chairs: Andreas Beyer, Darla L. Tharp

T-LII1-1 Eryn P. Wagoner[1,3], Shannon E. Draper[1,3], Pamela K. Thorne[1,3], Taylor J. Kelty[2,3], Michael S. Kapiloff[4], **Darla L. Tharp[1,3]** **15+5'**
[1]Biomedical Sciences, University of Missouri, Columbia, Missouri, United States
[2]Nutrition and Exercise Physiology, University of Missouri, Columbia, Missouri, United States
[3]NextGen Precision Health, University of Missouri, Columbia, Missouri, United States
[4]Ophthalmology and Medicine, Stanford Cardiovascular Institute, University of Stanford, Stanford, California, United States

16-Pathological adaptations of cerebral vasculature in a female Ossabaw swine model of cardiometabolic heart failure with preserved ejection fraction

T-LII1-2 **Calum Wilson**[1], John G McCarron, Matthew D Lee, Ross Steveson[1], Charlotte Buckley[1], Xun Zhang[1] **15+5'**
[1]Strathclyde Institute of Pharmacy & Biomedical Sciences, University of Strathclyde, Glasgow, United Kingdom
17-Mitochondrial ATP is necessary for physiological vascular function

T-LII1-3 Patricia E. McCallinhart[1], Kathlyene R. Stone[1], Pamela A. Lucchesi[2], Samir N. Ghadiali[3,4], **Aaron J. Trask**[1,5] **15+5'**
[1]Center for Cardiovascular Research, The Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, Ohio, United States
[2]University of Texas Tyler School of Medicine, Tyler, Texas, United States
[3]Department of Biomedical Engineering, The Ohio State University, Columbus, Ohio, United States
[4]Division of Pulmonary, Critical Care, and Sleep Medicine, Department of Internal Medicine, The Ohio State University Wexner Medical Center, Columbus, Ohio, United States
[5]Department of Pediatrics, College of Medicine, The Ohio State University Wexner Medical Center, Columbus, Ohio, United States
18-Coronary microvascular mechanics and blood flow in type 2 diabetes

T-LII1-4 **Petra Kleinbongard 15+5'**
Institute for Pathophysiology, West German Heart and Vascular Center, University of Essen Medical School, University of Duisburg-Essen, Essen, Germany
19-Damage and potential protection of the coronary microcirculation during myocardial infarction

10:50-11:20 **Coffee break and industrial exhibition**

11:20-12:40 **T-C3** **Coronary artery disease, coronary microvascular dysfunction and heart failure in women and men 80'**
Chair: Francesco Tona

T-C3-1 **Francesco Tona 15+5'**
University of Padova, Italy
21-Autoimmunity and coronary microvascular dysfunction: new actors in acute myocardial infarction?

T-C3-2 **Mengnan Wan**[1], Xiaoling Li[1,2], Benedikt Preckel[1], Markus W. Hollman[1], Coert J. Zuurbier[1], Nina C. Weber[1] **15+5'**

[1]Amsterdam, University Medical Centers, location AMC, Department of Anesthesiology – Laboratory of Experimental Intensive Care and Anesthesiology - L.E.I.C.A. Amsterdam Cardiovascular Science (ACS), Amsterdam, The Netherlands
[2]Center for cell lineage and development, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China

22-Empagliflozin prevents oscillatory shear stress-induced oxidative stress in human coronary artery endothelial cells via inhibition of NHE1/NCX

T-C3-3 **Giovanni Riccardo Maria Civieri 15+5'**
University of Padova, Italy
23-Mental stress and coronary microvascular dysfunction: a new brain-heart axis?

11:20-12:40 **T-LI2** **Specialized functions of the brain microvessels: from development to aging 80'**
Chairs: Mária Deli, István Krizbai

T-LI2-1 Amaia Dominguez-Belloso[1], Ricardo Figueiredo[2], Moritz Armbrust[1], Katharina Gneiding[1], Jennifer Krawietz[1], Natalie Hellmann[1], Sonja Thom[1], Sylvaine Guérit[1], Daniel Spitzer[1], Nienke M. deWit[3], Kavi Devraj[1], Peter Winter[2], Elga deVries[3], **Stefan Liebner[1] 20+5'**
[1]Institute of Neurology (Edinger-Institute), Goethe-University Clinic Frankfurt, Heinrich-Hoffmann-Straße 7, 60528 Frankfurt, Germany
[2]GenXPro GmbH, Altenhöferallee 3, D-60438 Frankfurt, Germany
[3]MS Center Amsterdam, Department of Molecular Cell Biology and Immunology, Amsterdam Neuroscience, Vrije Universiteit Amsterdam, Amsterdam UMC, De Boelelaan 1117, 1081 Amsterdam, the Netherlands
24-Amyloid-β effects on the blood-brain barrier: contribution to Alzheimer’s disease

T-LI2-2 **Fruzsina Walter**, Judit P. Vigh, Anna Kocsis, Nóra Kucsápszky, András Dér, Mária Deli **15+5'**
Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary
25-Engineering microfluidic chip models of the blood-brain barrier

T-LI2-3 Duc Lam Tri[1,2], István Tóth[1,3], Imola Wilhelm[1,4], Claudine Kieda[5,6], István Krizbai[1,7,4], **Attila Farkas[1] 15+5'**
[1]HUN-REN Biological Research Centre, Institute of Biophysics, Szeged, Hungary
[2]University of Szeged, Doctoral School of Biology, Szeged,

Hungary
[3]Szeged Scientists Academy, Foundation for the Future of Biomedical Sciences in Szeged, Szeged, Hungary
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[5]Military Institute of Medicine-National Research Institute, Laboratory of Molecular Oncology and Innovative Therapies, Warsaw, Poland
[6]CNRS, Centre for Molecular Biophysics, Orleans, France
[7]University of Szeged, Department of Cell Biology and Molecular Medicine, Szeged, Hungary
26-Senolysis potentiates endothelial progenitor cell adhesion to and integration into the brain vasculature

T-LI2-4 **Léa Ould Boukhitine**[1], Chloé Brizais[1], Florence Bachelot[1], Christelle Durand[1], Chrystelle Ibanez[1], Viriginie Monceau[1], Justyne Vetel[1], Imène Garali[1], Boris Manoury[2], Teni Ebrahimian[1] **10+5'**
[1]Experimental Radiotoxicology and Radiobiology Laboratory (LRTOX), Institute for Radiobiological Protection and Nuclear Safety (IRSN), Fontenay-aux-Roses, France
[2]Signaling and Cardiovascular Pathophysiology, Université Paris-Saclay, INSERM UMR-S 1180, Orsay, France
27-Effect of co-exposure to high-fat diet and low or moderate doses of ionizing radiation on the cerebral microcirculation

11:20-12:40 T-LII2 **Oxidative stress in microcirculation – dietary and environmental influences (a symposium of the Croatian Physiological Society) 80'**
Chairs: Ines Drenjančević, Ana Stupin

T-LII2-1 **Ana Stupin**[1], Ines Drenjančević[1], Zrinka Mihaljević[1], Ivana Jukić[1], Nikolina Kolobarić[1] **15+5'**
[1]Department of Physiology and Immunology, Faculty of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia
28-High salt diet increases oxidative stress and alters vasodilation in human microcirculation

T-LII2-2 **Helena Lenasi**[1], Lana Kralj[1] **15+5'**
[1]Institute of Physiology, Faculty of Medicine, University of Ljubljana, Slovenia
29-The effect of acute hyperglycemia on skin microvascular reactivity evaluated by an integrated approach

T-LII2-3 **Ivana Jukić**[1], Martina Kos[2,3], Tihana Nađ[2,3], Ana Stupin[1], Zrinka Mihaljević[1], Nikolina Kolobarić[1], Petar Šušnjara[4], Ines Drenjančević[1] **15+5'**

[1]Department of Physiology and Immunology, Faculty of Medicine Osijek, Osijek, Croatia
[2]Clinic of Pediatrics, University Hospital Centre Osijek, Osijek, Croatia
[3]Department of Pediatrics, Faculty of Medicine Osijek, Osijek, Croatia
[4]Faculty of Kinesiology Osijek, Osijek, Croatia
30-Microvascular function in children with hypertension

T-LII2-4 **Zrinka Mihaljević**[1], Ana Stupin[1], Ivana Jukić[1], Nikolina Kolobarić[1], Petar Šušnjara[2], Vedran Đambić[1], Aleksandar Kibel[1,3], Ines Drenjančević[1] **15+5'**
[1]Department of Physiology and Immunology, Faculty of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia
[2]Faculty of Kinesiology Osijek, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia
[3]International Medical Center Priora, Čepin, Croatia
31-Intermittent hyperbaric oxygenation restores the mechanisms of vasodilation in rat animal model

12:40-13:40 **Lunch break and Poster viewing**

13:40-14:30 **T-C4** **KEYNOTE LECTURE – Stefan Offermanns 50'**
Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany and Center for Molecular Medicine, Goethe University Frankfurt, Frankfurt, Germany
32-Microvascular functions in postischemia neovascularisation and type 2 diabetes
Chair: Zoltán Benyó

14:30-15:50 **T-C5** **Coronary microvascular function in health and disease 80'**
Chairs: Ákos Koller, Myriam Grunewald

T-C5-1 **Dirk J. Duncker 15+5'**
Erasmus University Medical Center, Rotterdam, The Netherlands
33-Coronary microvascular function in health & ischemic heart disease

T-C5-2 **Zsolt Bagi 15+5'**
Augusta University, Augusta GA, United States
34-Coronary microvascular dysfunction in metabolic disease

T-C5-3 **Myriam Grunewald,** Eli Keshet **15+5'**
Department of Developmental Biology and Cancer Research, Faculty of Medicine, The Hebrew University of Jerusalem, Israel
Hadassah Organoid Center, The Hadassah Medical Organization, Jerusalem, Israel

35-Microvessels, macro impact: The role of capillaries in jealthy aging

T-C5-4 **Nazha Hamdani 15+5'**
Ruhr University, Bochum, Germany
36-Inflammation and oxidative stress in endothelial-cardiomyocyte interactions: insights into heart failure with preserved ejection fraction

14:30-15:50 **T-LI3** **The cerebral circulation: the vascular contribution to dementia 80'**
Chairs: Erik N.T.P. Bakker, Leon Munting

T-LI3-1 **Anna Csiszar 15+5'**
Oklahoma Center for Geroscience and Healthy Brain Aging, Department of Neurosurgery, University of Oklahoma Health Sciences Center, Oklahoma City, United States
37-Age-related vascular cognitive impairment: role of endothelial senescence

T-LI3-2 **Leon Munting**[1], Mariel Kozberg[2], Orla Bonnar[2], Susanne van Veluw[2] **15+5'**
[1]Department of Radiology, Leiden University Medical Center, Leiden, Netherlands
[2]Department of Neurology, Massachusetts General Hospital, Boston, MA, United States
38-Vascular function and brain clearance measurements in a mouse model of cerebral amyloid angiopathy

T-LI3-3 **Marleen Veit**[1,2,3,4,5,6], Jessica Wagner[1,2,3,4,6], Karoline Degenhardt[1,3,4,], Nikolaos Louros[7,8], Katerina Konstantoulea[7,8], Angelos Skodras[1,3], Katleen Wild[1], Ping Liu[1,3,4], Ulrike Obermüller[3], Vikas Bansal[1], Anupriya Dalmia[1], Lisa M. Häsler[1,3], Marius Lambert[1,3], Matthias De Vleeschouwer[7,8], Hannah A. Davies[9,10], Jillian Madine[9,10], Deborah Kronenberg-Versteeg[3], Regina Feederle[1,12], Domenico Del Turco[13], K. Peter R. Nilsson[14], Tammaryn Lashley[15,16], Thomas Deller[13], Marla Gearing[17], Lary C. Walker[18], Peter Heutink[1], Frederic Rousseau[7,8], Joost Schymkowitz[7,8], Mathias Jucker[1,3], Jonas J. Neher[1,2,3,6] **15+5'**
[1]German Center for Neurodegenerative Diseases (DZNE), Germany
[2]Metabolic Biochemistry, Biomedical Center, LMU Munich, Munich, Germany
[3]Department of Cellular Neurology, Hertie Institute for Clinical Brain Research, University of Tübingen, Germany
[4]Graduate School of Cellular and Molecular Neuroscience, University of Tübingen, Germany
[5]Graduate School of Systemic Neurosciences, LMU Munich, Munich, Germany

[6]Munich Cluster for Systems Neurology (SyNergy), Munich, Germany
[7]Switch Laboratory, VIB-KU Leuven Center for Brain and Disease Research, Leuven, Belgium
[8]Switch Laboratory, Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium
[9]Department of Cardiovascular and Metabolic Medicine, Institute of Life Course and Medical Sciences, University of Liverpool, United Kingdom
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[17]Department of Pathology and Laboratory Medicine and Department of Neurology, Emory University School of Medicine, United States
[18]Department of Neurology and Emory National Primate Research Center, Emory University, Atlanta, United States

39-Medin amyloid and its role in Alzheimer's disease and vascular aging: A potential therapeutic target

T-LI3-4 **Shereen Nizari**[1,2], Marina Basalay[3], Pippa Chapman[2], Nils Korte[2], Alla Korsak[2], Isabel N. Christie[2], Shefeeq M. Theparambil[2], Sean M. Davidson[3], Frank Reimann[4], Stefan Trapp[2], Derek M. Yellon[3], Jack A. Wells[1], Mark Lythgoe[1], Alexander V. Gourine[2] **15+5'**
[1]Centre for Advanced Biomedical Imaging, University College London, London, United Kingdom
[2]Centre for Cardiovascular and Metabolic Neuroscience, University College London, London, United Kingdom
[3]The Hatter Cardiovascular Institute, University College

London, London, United Kingdom
[4]Wellcome Trust/MRC Institute of Metabolic Science, University of Cambridge, Cambridge, United Kingdom
40-GLP-1 and the cerebrovasculature

14:30-15:50	**T-LII3**	**Novel insights into microcirculation: from analytical approach to clinical applicability (a symposium of the Slovenian Physiological Society) 80'** Chairs: Helena Lenasi, Henrique N. Silva
	T-LII3-1	**Martin Hultman**[1,2], Joakim Henricson[3], Hugo Gustavsson[3], Tomas Strömberg[1], Marcus Larsson[1], Ingemar Fredriksson[1,2], Fredrik Iredahl[4], Rani Toll[3], Daniel Wilhelms[3] **15+5'** [1]Department of Biomedical Engineering, Linköping University, Sweden [2]Perimed AB, Järfälla-Stockholm, Sweden [3]Department of Emergency Medicine in Linköping, and Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden [4]Primary Health Care Center, Department of Health, Medicine and Caring Sciences, Faculty of Medicine and Health Sciences, Linköping University, Linköping, Sweden *41-Imaging microcirculation in a controlled in-vivo model of sepsis*
	T-LII3-2	**Lana Kralj**[1], Helena Lenasi[1] **15+5'** [1]Institute of Physiology, Faculty of Medicine, University of Ljubljana, 1000 Ljubljana, Slovenia *42-Advancing skin microvascular assessment: A comparative review of wavelet analysis*
	T-LII3-3	**Henrique Silva**[1,2,3], Carlota Rezendes[2] **15+5'** [1]Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal [2]Department of Pharmacy, Pharmacology and Health Technologies, Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, Lisbon, Portugal [3]Biophysics and Biomedical Engineering Institute (IBEB), Faculdade de Ciências, Universidade de Lisboa, Campo Grande, Lisbon, Portugal *43-Exploring microvascular reactivity with photoplethysmography*
	T-LII3-4	**Ines Drenjančević 15+5'** Department of Physiology and Immunology, Faculty of Medicine Osijek, Osijek, Croatia *44-Microvascular reactivity in chronic kidney disease in human*

15:50-16:20		**Coffee break and industrial exhibition**

16:20-17:40 T-C6 **The role of microvasculature in cardiovascular system and its assessment in the office of angiologist 80'**

Chairs: Jan Pitha, Petra Korpisalo

T-C6-1 **Petra Korpisalo**[1,2], Galina Wirth[1,3], Greta Juusola[1,3], Santeri Tarvainen[1,3], Crister Frimodig[1], Krista Happonen[1], Seppo Ylä-Herttuala[3,1], Juhani Knuuti[4], Harri Hakovirta[5], Kimmo Mäkinen[1] **15+5'**
[1]Heart Center, Kuopio University Hospital, Kuopio, Finland
[2]Department of Clinical Medicine, University of Eastern Finland, Kuopio, Finland
[3]A.I. Virtanen Institute, University of Eastern Finland, Kuopio, Finland
[4]Turku PET Centre, Turku University Hospital, Turku, Finland
[5]Department of Vascular Surgery, Turku University Hospital, Turku, Finland
45-Microvascular over-dilation - a novel tissue level mechanism causing hypoxia and chronic tissue damage with reference also to skeletal muscle regeneration

T-C6-2 **Riikka Kivelä**[1,2,3] **15+5'**
[1]Faculty of Sport and Health Sciences, University of Jyväskylä, Finland
[2]Wihuri Research Institute, Helsinki, Finland
[3]Stem Cells and Metabolism Research Program, Faculty of Medicine, University of Helsinki, Helsinki, Finland
46-The cardiac microvasculature in aging, obesity and exercise

T-C6-3 **Jan Pitha 15+5'**
Institute for Clinical and Experimental Medicine, Prague, Czech Republic
47-Circulatory disorders in type 1 diabetes type, from big to small vessels and vice versa

T-C6-4 **Jiri Matuska 15+5'**
Matuska J, MATMED s.r.o., Hodonin, Czech Republic
48-Diabetes related microangiopathy assessment in the angiologist everyday practice

16:20-17:40 T-LI4 **Advances in cerebromicrovascular aging: mechanisms and therapeutic interventions 80'**

Chairs: Stefano Tarantini, Zoltan Ungvari

T-LI4-1 **Sharon Negri**[1,2], Adam Nyul-Toth[1], Madison Milan[1,2], Eva Troyano-Rodriguez[1,2], Sherwin Tavakol[1,2], Jennifer Ihuoma[1,2], Zeke Reyff[1,2], Rakesh Rudraboina[1,2], Rafal Gulej[1], Anna Csiszar[1], Zoltan Ungvari[1], Stefano Tarantini[1,2] **15+5'**

[1]Vascular Cognitive Impairment and Neurodegeneration Program, Reynolds Oklahoma Center on Aging/Center for Geroscience and Healthy Brain Aging, Dept. of Neurosurgery, OUHSC, OKC, OK, United States
[2]Stephenson Cancer Center, OUHSC, OKC, OK, United States

49-Longitudinal functional ultrasound (fUS) imaging reveals the impact of dietary choices on cerebrovascular aging

T-LI4-2 **Roland Patai**[1,2], Krish Patel[1], Adam Nyul-Toth[1,2], Boglarka Csik[1,2], Rafal Gulej[1,2], Raghavendra Y. Nagaraja[1,2], Dorina Nagy[1,2], Siva S. Chandragiri[1,2], Santny Shanmugarama[1,2], Kiana V. Kordestan[1,2], Mark Nagykaldi[1,2], Shoba Ekambaram[1,2], Anna Ungvari[3], Anna Csiszar,[1,2,4], Zoltan Ungvari[1,2,4] **15+5'**
[1]Vascular Cognitive Impairment, Neurodegeneration, and Healthy Brain Aging Program, Department of Neurosurgery, University of Oklahoma Health Sciences Center, Oklahoma City, OK, Unites States
[2]Oklahoma Center for Geroscience and Healthy Brain Aging, University of Oklahoma Health Sciences Center, Oklahoma City, OK, Unites States
[3]Institute of Preventive Medicine and Public Health, Semmelweis University, Budapest, Hungary
[4]The Peggy and Charles Stephenson Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK, Unites States

50-Targeting cellular senescence to prevent cerebral microhemorrhages in aging

T-LI4-3 **Szilvia Kecskes**[1,2], Ákos Menyhárt[1,2], Eszter Farkas[1,2] **15+5'**
[1]HCEMM-USZ Cerebral Blood Flow and Metabolism Research Group, HCEMM Nonprofit Ltd., Szeged, Hungary
[2]Department of Cell Biology and Molecular Medicine, University of Szeged, Szeged, Hungary

51-Senolytic therapy with Dasatinib and Quercetin mitigates focal stroke outcomes in the aging rat brain

T-LI4-4 **Tamás Csípő**[1,2], Ágnes Lipécz[1,2], Péter Mukli[1,2], Anna Ungvári[1], Attila Kállai[1], Stefano Tarantini[2], Anna Csiszár[2], Zoltán Benyó[3], Ádám G. Tabák[1,4,5], Andriy Yabluchanskiy[2], Zoltán Ungvári[1,2] **15+5'**
[1]Department of Public Health, Faculty of Medicine, Semmelweis University, Budapest, Hungary
[2]Vascular Cognitive Impairment, Neurodegeneration and Healthy Brain Aging Program, Department of Neurosurgery, University of Oklahoma Health Sciences Center, Oklahoma City, OK, United States
[3]Department of Translational Medicine, Semmelweis University, Budapest, Hungary

[4]UCL Brain Sciences, University College London, London, United Kingdom
[5]Department of Internal Medicine and Oncology, Semmelweis University, Faculty of Medicine, Budapest, Hungary
52-Development of a comprehensive vascular assessment for a prospective workplace base cohort (The Semmelweis Study): contribution of retinal and peripheral vascular dysfunction to age-related vascular cognitive impairment

16:20-17:40 **T-LII4** **Health and racial disparities in vascular physiology 80'**
Chairs: Amanda J. Leblanc, Chantal Eickelmann

T-LII4-1
Shelby N. Hader[1,2], Erin C. Birch[1,2], Yoshinori Nishijima[1,2], Laura Norwood Toro[1,2], Janée D. Terwoord[6], Amanda Kong[4,5], Caitlin Patten[4,5], David D. Gutterman[1,2,3] **Andreas M. Beyer[1,2,3]** **15+5'**
[1]Department of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin, United Stated
[2]Cardiovascular Center, Medical College of Wisconsin, Milwaukee, Wisconsin, United Stated
[3]Department of Physiology, Medical College of Wisconsin, Milwaukee, Wisconsin, United Stated
[4]Department of Surgery, Medical College of Wisconsin, Milwaukee, Wisconsin, United Stated
[5]Department of Oncology Medical College of Wisconsin, Milwaukee, Wisconsin, United Stated
[6]Department of Biomedical Sciences, Rocky Vista University, Ivins, Utah, United States
53-Racial disparities and microvascular function in cardio-oncology

T-LII4-2
Elien Heylen[1,*], Jozafina Haj-Shomaly[1,*], Anisha Thind[2], Eleni Maniati[2], Anna E. A. Surace[3], Katie Baird[2], Ryan Wallis[4], Luke Gammon[4], Isobelle Wall[5], Jelmar Quist[5], Rachel Nelan[2], Thamid Yaseen[2], Rachel Barrow[2], Naushin Waseem[2], Catherine McMaster-Christie[2], Noorul Wahab[6], Myles J. Lewis[3], Nasir Rajpoot[6], Anita Grigoriadis[5], Jun Wang[2], Louise Jones[2,&], **Gabriela D'Amico[1]**, Kairbaan Hodivala-Dilke[1] **15+5'**
[1]Centre for Tumour Microenvironment, a Cancer Research United Kingdom of Excellence, Queen Mary University of London, Charterhouse Square, London, United Kingdom
[2]Barts Cancer Institute, a Cancer Research United Kingdom of Excellence, Queen Mary University of London, Charterhouse Square, London, United Kingdom
[3]Centre for Translational Bioinformatics, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom

[4]Phenotypic Screening Facility, Blizard Institute, Faculty of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom
[5]School of Cancer & Pharmaceutical Sciences, Faculty of Life Sciences and Medicine, King's College London, London, United Kingdom
[6]Tissue Image Analytics Centre, Department of Computer Science, University of Warwick, Coventry, United Kingdom

54-Spatial mapping of breast cancer tumour microenvironment in Black British and White British women

T-LII4-3 **Styliani Goulopoulou**[1], Nataliia Hula[1], Renée de Nazaré Oliveira da Silva[1], Jessica L. Bradshaw[2], Spencer C. Cushen[2], Nicole R. Phillps[3] **15+5'**

[1]Lawerence D. Longo, MD Center for Perinatal Biology, Loma Linda University, Loma Linda, California, United States
[2]Department of Physiology and Anatomy, University of North Texas Health Science Center, Fort Worth, Texas, United States
[3]Department of Microbiology, Immunology & Genetics, University of North Texas Health Science Center, Fort Worth, Texas, United States

55- Activation of the mtDNA-TLR9 pathway induces hypertension and endothelial dysfunction in pregnant rats, modelling key features of preeclampsia

T-LII4-4 **Suvi Linna-Kuosmanen** **15+5'**

A.I.Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland

56-Piece of Heart - Piecing together cardiovascular disease mechanisms from ex vivo human cardiac tissue using multi-omics

17:40-19:00 **General Assembly**

May 21, 2025 Wednesday

8:30-9:20	**W-C1**	**KEYNOTE LECTURE – Mohamad El Amki 50'** University Hospital of Zurich, Zurich, Switzerland *57-Exploring the brain vascular function: from the pial vasculature to the delicate capillaries* <u>Chair:</u> Ákos Menyhárt
9:30-10:50	**W-C2**	**The no-reflow phenomenon after acute ischemic stroke: players and mechanistic insights 80'** <u>Chairs:</u> Ákos Menyhárt, Anna Törteli
	W-C2-1	Christian Staehr[1,2], **John T. Giblin**[2], Halvor Guldbrandsen[1], Eugenio Gutiérrez-Jiménez[3], David Boas[2], Vladimir Matchkov[1] **15+5'** [1]Department of Biomedicine, Aarhus University, Denmark [2]Neurophotonics Center, Department of Biomedical Engineering, Boston University, Boston, MA, United States [3]Center of Functionally Integrative Neuroscience, Aarhus University, Denmark *58-Neurovascular uncoupling is linked to microcirculatory dysfunction in regions outside the ischemic core following ischemic stroke*
	W-C2-2	**Chaim Glück**[1,2], Jeanne Droux[2,3], Susanne Wegener[2,3], Bruno Weber[1,2], Mohamad El Amki[2,3] **15+5'** [1]Institute of Pharmacology and Toxicology, University of Zurich, Zurich, Switzerland [2]ZNZ Neuroscience Center, University and ETH Zurich, Zurich, Switzerland [3]Department of Neurology, University Hospital Zurich, Zurich, Switzerland *59-Distal embolization during clot dissolution as contributor to the no-reflow phenomenon*
	W-C2-3	**Joshua James Shrouder,**[1,2] Gian Marco Calandra,[1] Severin Filser,[1,2,3] Daniel Peter Varga,[1,2] Simon Besson-Girard,[1,2] Uta Mamrak, Maximilian Dorok, Buket Bulut-Impraim,[1,2] Fatma Burcu Seker,[1,2] Benno Gesierich,[1,2] Fabio Laredo,[1] Antonia Clarissa Wehn,[1,2,4] Igor Khalin,[1,2,5] Patrick Bayer,[1] Arthur Liesz,[1,2] Ozgun Gokce[1,2] and Nikolaus Plesnila[1,2] **15+5'** [1]Institute for Stroke and Dementia Research (ISD), LMU University Hospital, Ludwig-Maximilians-University (LMU), Munich, 81377 Munich, Germany [2]Munich Cluster for Systems Neurology (SyNergy), 81377 Munich, Germany [3]Core Research Facilities and Services—Light Microscope Facility, German Center for Neurodegenerative Diseases, (DZNE), 53127 Bonn, Germany

[4]Department of Neurosurgery, LMU University Hospital, Ludwig-Maximilians-University (LMU) Munich, 81377, Munich, Germany
[5]Normandie University, UNICAEN, INSERM UMR-S U1237, Physiopathology and Imaging of Neurological Disorders, (PhIND), GIP Cyceron, Institute Blood and Brain @ Caen-Normandie (BB@C), 14000 Caen, France
60-Continued dysfunction of capillary pericytes promotes no-reflow after experimental stroke in vivo

W-C2-4 **Ákos Menyhárt[1,2]**, Anna Törteli[1,2], Réka Tóth[1,2], Ferenc Bari[3], Eszter Farkas[1,2] **15+5'**
[1]Hungarian Centre of Excellence for Molecular Medicine – University of Szeged Cerebral Blood Flow and Metabolism Research Group, Szeged, Hungary
[2]Department of Cell Biology and Molecular Medicine, Albert Szent Györgyi Medical School and Faculty of Science and Informatics, University of Szeged, Szeged, Hungary
[3]Department of Medical Physics and Informatics, Albert Szent-Györgyi Medical School and Faculty of Science and Informatics, University of Szeged, Szeged, Hungary
61-Collateral is brain: Low perfusion triggers spreading depolarization and futile reperfusion after acute ischemic stroke

9:30-10:50 **W-LI1** **Fundamental and clinical microcirculation and hemorheology 80'**
Chairs: Maka Manstkava, Nana Momtselidze

W-LI1-1 **Maka Mantskava**, Giorgi Kuchava, Nana Momtselidze **15+5'**
Department of Rheology and Diagnostic Analytical Services, Ivane Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia
62-About microcirculation and hemorheology in leukemia

W-LI1-2 **Maia Gotsadze[1]**, Eka Arziani[1], Tamar Kandashvili[1], Nana Momtselidze[2,3], Maka Mantskava[1,2,3] **15+5'**
[1]Tbilisi State Medical University, Tbilisi, Georgia
[2]Society of Rheologist, Tbilisi, Georgia, Ivane Beritahvili Center of Biomedicine, Tbilisi, Georgia
[3]Ivane Beritashvili Experimental Center of Biomedicine, Tbilisi, Georgia
63-Hemorheologycal study in patients with different form of atrial fibrillation

W-LI1-3 Nana Momtselidze[1,2,] **Tamar Urdulashvili[3]**, Maka Mantskava[1,4] **15+5'**
[1]Ivane Beritashvili Experimental Center of Biomedicine, Tbilisi, Georgia
[2]Central University of Europe, Kutaisi, Georgia

[3]High Technology Medical Centre, University Clinic
[4]European University, Tbilisi, Georgia
64-Resistive index for studying microcirculation

W-LI1-4 **Keith Afas**[1], Daniel Goldman[2] **15+5'**
[1]Department of Biomedical Engineering, University of Western Ontario, London, Canada
[2]Department of Medical Biophysics, University of Western Ontario, London, Canada
65-Development of a hybrid fluid-structure interaction (FSI) algorithm for red blood cell microcirculatory deformation under shear stress, and hypothesized O_2-dependent ATP release

9:30-10:50 **W-LII1** **Restoring microcirculation by natural compounds by improving cellular energy metabolism 80'**
Chairs: Jing-Yan Han, Jian-Gang Shen

W-LII1-1 Zheng Wang[1,2], Jing-Yan Han[1,2], **Jian Liu**[1] **15+5'**
[1]Department of Integration of Chinese and Western Medicine, School of Basic Medical Sciences, Peking University, Beijing, China
[2]Tasly Microcirculation Research Center, Peking University Health Science Center, Beijing, China
66-QiShenYiQi pills inhibited retinal microvascular hyperpermeability in db/db mice

W-LII1-2 **Jiangang Shen**, Ziqiao Xu, Qiaohui Du **15+5'**
School of Chinese Medicine, State Key Laboratory of Pharmaceutical Biotechnology, The University of Hong Kong, Hong Kong, China
68-Ginseng and Panax Notoginseng formula promotes post-stroke neurogenesis and neurological functional recovery through improving mitochondrial function and modulating oxidative redox signaling pathway

W-LII1-3 *Dan-Tong Li[1,2], Ayididaer Ayan[1,2], **Kai Sun**[2], Chun-Shui Pan[2], Li Yan[2], Ping Huang[2], Jing-Yan Han[1,2]* ***15+5'***
[1]Department of Integration of Chinese and Western Medicine, School of Basic Medical Sciences, Peking University, Beijing, China
[2]Tasly Microcirculation Research Center, Peking University Health Science Center, Beijing, China
69-Traditional Chinese medicine Yiqifumai injection ameliorates LPS-induced microvascular hyperpermeability in multiple organs through energy metabolism

10:50-11:20 **Coffee break and industrial exhibition**

11:20-12:40 **W-C3** **The post-stroke brain microcirculation: From ion channels to local reperfusion 80'**
Chairs: Ed van Bavel, Vladimir V. Matchkov

W-C3-1 Elizaveta Melnikova[1], Ida Damsgard Larsen[1], Eugenio Gutierrez[1,2], Hans Christian Beck[3], Christian Aalkjaer[1], Dmitry D. Postnov[2], Line Mathilde Brostrup Hansen[1], **Vladimir V. Matchkov[1] 15+5'**
[1]Department of Biomedicine, Aarhus University, Aarhus, Denmark
[2]Center of Functionally Integrative Neuroscience, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark
[3]Department of Clinical Biochemistry, Odense University Hospital, Odense, Denmark
70-Chloride channel, TMEM16A in stroke

W-C3-2 **Line Mathilde Brostrup Hansen[1]**, Vibeke Secher Dam[1], Halvor Østerby Guldbrandsen[1], Christian Staehr[1,4], Tina Myhre Pedersen[1], Joanna Maria Kalucka[1], Hans Christian Beck[2,3], Dmitry D. Postnov[4], Lin Lin[1], Vladimir V. Matchkov[1] **15+5'**
[1]Department of Biomedicine, Aarhus University, Aarhus, Denmark
[2]Institute of Clinical Research, University of Southern Denmark, 5000 Odense, Denmark
[3]Centre for Clinical Proteomics, Department of Clinical Biochemistry, Odense University Hospital, 5000 Odense, Denmark
[4]Department of Anaesthesiology and Intensive Care, Aarhus University Hospital, Aarhus, Denmark
71-Blood-brain barrier disruption and neurovascular uncoupling after stroke

W-C3-3 ***Kevin Mol[1]***, *Elga de Vries[2], Ed van Bavel[1], Inge Mulder[1]* **15+5'**
[1]Biomedical Engineering & Physics, Amsterdam University Medical Center, Amsterdam, Netherlands
[2]Molecular Cell Biology & Immunology, Amsterdam University Medical Center, Amsterdam, Netherlands
72-Microvascular recanalization by embolus extravasation in a rodent model of silent brain infarcts

W-C3-4 Chryso Lambride[1,2,3], Gaia Stievano[1], Timo Koch[4], Mohamad El Amki[2,3], Susanne Wegener[2,3], **Franca Schmid[1] 15+5'**
[1]ARTORG Center, University of Bern, Bern, Switzerland
[2]Department of Neurology, University Hospital & University of Zurich, Zurich, Switzerland
[3]Neuroscience Center Zurich, University of Zurich & ETH Zurich, Zurich, Switzerland
[4]Scientific Computing and Numerical Analysis, Simula Research Laboratory, Oslo, Norway

73-Stroke across scales – Impact on blood perfusion and oxygen supply in the brain

11:20-12:40 **W-LI2** **Emerging models and mechanisms of barrier regulation 80'**

Chairs: Cristina Branco, Szilvia Veszelka

W-LI2-1 **Anikó Szecskó**[1,2], Koppány Párdi[1], Zuhao Cui[1,2], Nárcisz Cser[1], Gergő Porkoláb[1], Janet Adegbite[1], Mária A. Deli[1], Szilvia Veszelka[1] **14+2'**

[1]Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary

[2]Doctoral School of Biology, University of Szeged, Szeged, Hungary

126- The histone deacetylase inhibitor suberoylanilide hydroxamic acid promotes blood-brain barrier protection during reperfusion in a cell culture model of ischemic stroke

W-LI2-2 **Jovana Serbanovic-Canic 20+3'**

Division of Clinical Medicine, University of Sheffield, Sheffield, United Kingdom

75-The role of polycystin-1 in endothelial dysfunction and cardiovascular disease

W-LI2-3 **Shakira A. van der Panne**[1,3,7], Elga de Vries[2,3,4], Louise van der Weerd[5,6], Gustav J. Strijkers[1,7], Erik N.T.P. Bakker[1] **14+2'**

[1]Department of Biomedical Engineering & Physics, Amsterdam University Medical Center, Amsterdam, the Netherlands

[2]Department of Molecular Cell Biology and Immunology, Amsterdam University Medical Center, Amsterdam, the Netherlands

[3]Amsterdam Neuroscience, Amsterdam University Medical Center, Amsterdam, the Netherlands

[4]MS Center Amsterdam, Amsterdam University Medical Center, Amsterdam, the Netherlands

[5]Department of Radiology, Leiden University Medical Center, Leiden, the Netherlands

[6]Department of Human Genetics, Leiden University Medical Center, Leiden, the Netherlands

[7]Amsterdam Cardiovascular Sciences, Amsterdam University Medical Center, Amsterdam, the Netherlands

76-Ultrasound and microbubble-induced blood-brain barrier opening: structural integrity and permeability assessment

W-LI2-4 **Matthew Campbell 20+3'**

Trinity College Dublin, Ireland

77-Claudin-5 regulation at the blood brain barrier in health and disease

11:20-12:40 **W-LII2** **Microvascular highpermeability and regulation of traditional Chinese medicine 80'**

Chairs: Qiao-Bing Huang, Jing-Yan Han

W-LII2-1 **Qiaobing Huang**[1,2]**,** Maomao Sun[1,2], Zhenhua Zeng[2], Zhiya Deng[2], Jie Wu[2], Ruimin Lu[1], Gege Xu[2], Zhongqing Chen[2] **15+5'**
[1]Department of Pathophysiology, Guangdong Provincial Key Laboratory of Cardiac Function and Microcirculation, School of Basic Medical Sciences, Southern Medical University, Guangzhou, China
[2]Department of Critical Care Medicine, Nanfang Hospital, Southern Medical University, Guangzhou, China
78-The role of protein acetylation in organ dysfunction during sepsis and the effect of SIRTs

W-LII2-2 **Miao Wang 15+5'**
State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
79-Mechanisms of microvascular hyperpermeability and therapeutic targeting

W-LII2-3 **Akos Koller**[1, 2, 3, 4], Anna Júlia Éliás[1,5], Lili Zita Tersztyanszky[1], Dora Jarai[1] **15+5'**
[1]Department of Morphology and Physiology, HUN-REN-SE Cerebrovascular and Neurocognitive Disease Research Group, Semmelweis University, Budapest, Hungary
[2]Institute of Translational Medicine, HUN-REN-SE Cerebrovascular and Neurocognitive Disease Research Group, Semmelweis University, Budapest, Hungary
[3]Research Center for Sports Physiology, Hungarian University of Sports Science, Budapest, Hungary,
[4]Department of Physiology, New York Medical College, Valhalla, NY, United States
[5]Doctoral School, Health Sciences Division, Semmelweis University, Budapest, Hungary
80-Diet-induced improvements of microvascular endothelial function: role of walnuts in maintaining membrane integrity

W-LII2-4 **Jing-Yan Han**[1,2]
[1]Department of Integration of Chinese and Western Medicine, School of Basic Medical Sciences, Peking University, Beijing, China
[2]Tasly Microcirculation Research Center, Peking University Health Science Center, Beijing, China
81-The role and mechanism of traditional Chinese medicine in improving microvascular hyperpermeability

12:40-13:40 **Lunch break and Poster viewing**

13:40-14:30 W-C4 KEYNOTE LECTURE – Sarah Barman 50'

School of Computer Science and Mathematics, Kingston University, London, United Kingdom

82-Detection and quantification of vessel morphology on retinal fundus images – challenges of retinal vasculometry at scale

Chair: Alfons Houben

14:30-15:50 W-C5 Microcirculatory responses to spreading depolarisation in patients and animals 80'

Chairs: Jens P. Dreier, Rob Wykes

W-C5-1

Jens P. Dreier[1,2,3,4,5] **15+5'**
[1]Center for Stroke Research Berlin
[2]Department of Experimental Neurology, Charité – Universitätsmedizin Berlin, Berlin, Germany
[3]Department of Neurology, Charité – Universitätsmedizin Berlin, Berlin, Germany
[4]Bernstein Centre for Computational Neuroscience Berlin, Berlin, Germany
[5]Einstein Center for Neurosciences Berlin, Berlin, Germany

83-Microcirculatory responses to spreading depolarisation in patients during neurocritical care

W-C5-2

Samuel Flaherty[1,2], Lorena F. Fernandes[1,2], Masvidal-Codina[3,4], Xavier Illa[4,5], Elisabet Prats-Alfonso[4,5], Kostas Kostarelos[2], Stuart Allan[1], Jose Antonio Garrido[3], Anton Guimerà-Brunet[4,5], Rob C. Wykes[1,2,6] **15+5'**
[1]The Division of Neuroscience and the Brain Inflammation Group, University of Manchester, United Kingdom
[2]The Centre for Nanotechnology in Medicine, University of Manchester, United Kingdom
[3]Catalan Institute of Nanoscience and NanotechnologyICN2, CSIC and The Barcelona Institute of Science and Technology BIST, Campus UAB, Bellaterra, Barcelona, Spain
[4]Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina CIBER-BBN, Madrid, Spain
[5]Institut de Microelectrònica de Barcelona, IMB-CNMCSIC, Esfera UAB, Bellaterra, Spain
[6]Department of Clinical & Experimental Epilepsy, UCL Queen Square Institute of Neurology, United Kingdom

84-Spreading depolarisation (SD) waveform predicts SD-mediated localised haemodynamic responses in ischaemic brain tissue

W-C5-3

Lorena F. Fernandes[1,2], Samuel Flaherty[1,2], Masvidal-Codina[3,4], Xavier Illa[4,5], Eduard Prats-Alfonso[4,5], Kostas Kostarelos[2], Stuart

Allan[1], Jose Antonio Garrido[3], Anton Guimerà-Brunet[4,5], **Rob C. Wykes**[1,2,6] **15+5'**
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[5]Institut de Microelectrònica de Barcelona, IMB-CNMCSIC, Esfera UAB, Bellaterra, Spain
[6]Department of Clinical & Experimental Epilepsy, UCL Queen Square Institute of Neurology, United Kingdom
85-Ketamine inhibits the inverse haemodynamic response to spreading depolarisation in early focal cerebral ischemia

W-C5-4

Coline L. Lemale[1,2,] Lina M. Serna Higuita [3], Baptiste Balança [4,5], Sara Simula [6], Sebastian Major [1,2,7], Peter Martus [3], Ingemar Fredriksson [8,9], Jens Dreier [1,2,7] **15+5'**
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[8]Department of Biomedical Engineering, Linköping University, Sweden
[9]Perimed AB, Järfälla, Sweden
86-Effect of SIN-1 on the SD-initiated negative ultraslow potential and the no-reflow phenomenon during forebrain ischemia and reperfusion in rats

14:30-15:50 W-LI3

Symposium sponsored by Imedos: Retinal microvasculometry the tool for prediction and follow up of disease 80'
Chairs: Alfons Houben, Henner Hanssen

W-LI3-1 *Lennert Beeckmans[1,2], **Jan Van Eijgen[1,3]**, Nestor-Ventura-Abreu[1,4], Amaryllis Van Craenenbroeck[5,6], Maarten De Vos[2,7], Ingeborg Stalmans[1,3]* **15+5'**

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[7]Department of Development & Regeneration, KU Leuven, Leuven, Belgium

87- Improved noise reduction in dynamic vessel analysis using heartbeat filtering and RPCA

W-LI3-2 **Frank CT van der Heide[1,2]**, Ronald MA Henry[1,2,3], Alfons JHM Houben[1,2], Abraham A Kroon[1,2], Carla JH van der Kallen[1,2], Pieter C Dagnelie[1,2], Martien CJM van Dongen[4,5], Simone JPM Eussen[1,5], Tos TJM Berendschot[6], Jan SAG Schouten[7], Carroll AB Webers[6], Marleen MJ van Greevenbroek[1,2], Anke Wesselius[8], Annemarie Koster[4,9], Hans HCM Savelberg[10], Nicolaas C Schaper[1,2], Casper G Schalkwijk[1,2], Miranda T Schram[1,2,3,11], Seb Köhler[11,12], Martin PJ van Boxtel[11,12], Coen DA Stehouwer[1,2] **15+5'**

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[12]Department of Psychiatry and Neuropsychology, MUMC+ Maastricht, The Netherlands
88-Neurodegeneration, microvascular dysfunction and their interaction: associations with cognitive performance – The Maastricht Study

W-LI3-3 **Christoph Hauser**[1], Lukas Streese[1,2], Denis Infanger[1], Alice Minghetti[1], Henner Hanssen[1] **15+5'**
[1]Department of Sport, Exercise and Health, Medical Faculty, University of Basel, Basel, Switzerland
[2] Faculty of Health Care, Niederrhein University of Applied Sciences, Krefeld, Germany
89-Retinal vessels and blood pressure progression in children

W-LI3-4 **Doreen Schmidl**[1], Viktoria Pai[1], Andrea Bileck[2,3], Nikolaus Hommer[1], Patrick Janku[1], Theresa Lindner[1], Victoria Kauer[1,4], Benedikt Rumpf[4], Helmuth Haslacher[5], Gerhard Hagn[2], Samuel M Meier-Menches[2,3,6], Leopold Schmetterer[1,7-12], Christopher Gerner[2,3], Gerhard Garhöfer[1] **15+5'**
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[5]Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria
[6]Institute of Inorganic Chemistry, Faculty of Chemistry, University of Vienna, Vienna, Austria
[7]Singapore Eye Research Institute, Singapore National Eye Centre, Singapore, Singapore
[8]Ophthalmology and Visual Sciences Academic Clinical Program, Duke-NUS Medical School, Singapore, Singapore
[9]SERI-NTU Advanced Ocular Engineering (STANCE), Singapore, Singapore
[10]School of Chemistry, Chemical Engineering and Biotechnology, Nanyang Technological University, Singapore, Singapore
[11]Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria

[12]Institute of Molecular and Clinical Ophthalmology, Basel, Switzerland
90-Retinal microcirculation in patients that have recovered from acute COVID-19 infection

14:30-15:50 W-LII3 Microvascular diversity: from function to dysfunction 80'
Chair: Imola Wilhelm

W-LII3-1 **Christian Aalkjær**[1], Vladimir Matchkov[1], Vibeke Secher Dam[2] **15+5'**
[1]Department Biomedicine, Aarhus University, Denmark
[2]Department Clinical Medicine, Aarhus University, Denmark
91-Crosstalk between two calcium activated chloride conductances in the wall of small arteries

W-LII3-2 **Philippe Vangrieken**[1], Jean L.J.M. Scheijen[1], Etto C. Eringa[2], Casper G. Schalkwijk[1] **15+5'**
[1]School for Cardiovascular Diseases (CARIM), Department of Internal Medicine, Maastricht University Medical Center+, Maastricht, The Netherlands
[2]School for Cardiovascular Diseases (CARIM), Department of Physiology, Maastricht University Medical Center+, Maastricht, The Netherlands
92-Dietary methylglyoxal attenuates blood pressure, microvascular dysfunction, arterial stiffness and insulin resistance in high-fat diet-induced diabetic mice

W-LII3-3 **Zilin Sun**[1], Miao He[1], Yan Liu[1], Xiaoying Zhou[1], Shanhu Qiu[2] **15+5'**
[1]Department of Endocrinology, Zhongda Hospital, Institute of Diabetes, School of Medicine, Southeast University, Nanjing, China
[2]Department of General Practice, Zhongda Hospital, Institute of Diabetes, School of Medicine, Southeast University, Nanjing, China
93-Quantitive analysis of macular microvascular characteristics for diabetes and prediabetes using optical coherence tomography angiography

W-LII3-4 **Greta Juusola**[1,2], Galina Wirth[1,2], Tiit Örd[3], Minna Kaikkonen-Määttä[3], Harri Hakovirta[4], Seppo Ylä-Herttuala[2], Petra Korpisalo[1] **15+5'**
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[2]Group of Molecular medicine, A. I. Virtanen Institute, University of Eastern Finland, Kuopio, Finland
[3]Group of Cardiovascular Genomics, A. I. Virtanen Institute, University of Eastern Finland, Kuopio, Finland
[4]Department of Vascular Surgery, Turku University Hospital, Finland

		94-Differential gene expression of normal, enlarged and arterialized capillaries in ischemic skeletal muscle of patients with chronic limb threatening ischemia
15:50-16:20		**Coffee break and industrial exhibition**
16:20-18:00		**Guided poster session**
17:30-19:30	**W-C6**	**PUBLIC LECTURE – Ferenc Bari 120'** University of Szeged, Hungary Bepillantás a mikrocirkuláció rejtelmeibe
19:00-22:00		**Networking dinner**

May 22, 2025 Thursday

8:30-9:20	**TH-C1**	**KEYNOTE LECTURE – Kristina Haase 50'** European Molecular Biology Laboratory (EMBL), Barcelona, Spain *95-Engineered Vasculature – bridging human biology with translation* Chair: Mária Deli
9:30-10:50	**TH-C2**	**Vascular redox signalling and oxidative stress - From bench to bedside 80'** Chairs: Henning Morawietz, Katrin Schröder
	TH-C2-1	**Katrin Schröder 15+5'** Goethe University, Frankfurt, Germany *96-Vascular redox signalling und oxidative stress*
	TH-C2-2	**Henning Morawietz 15+5'** Division of Vascular Endothelium and Microcirculation, Department of Medicine III, Faculty of Medicine and University Hospital Carl Gustav Carus, TU Dresden, Germany *97-Redox signalling, oxidative stress, endothelial dysfunction and cardiometabolic diseases*
	TH-C2-3	**Richard Nosalski 15+5'** Centre for Cardiovascular Sciences, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, United Kingdom *98- Interplay between inflammation and oxidation in hypertension*
	TH-C2-4	**Bálint Bányai,** Stella Timea Kiss, Mária Szekeres Izabella Marton, Zsolt Papp, Ágnes Pakuts, Rita Benkő, Eszter Mária Horváth **15+5'** Semmelweis University Department of Physiology, Budapest, Hungary *99-Effect of vitamin D on vascular oxidative nitrative stress in a rat model of PCOS*
9:30-10:50	**TH-LI1**	**Translational medicine and microcirculation in surgical clinical practice 80'** Chairs: Béla Debreczeni, Ákos Koller
	TH-LI1-1	**Béla Zoltán Debreczeni 15+5'** DrD Plastic Surgery Studio, Budapest, Hungary *100-Plastic surgery procedures and wound edge microcirculation: Therapeutic options and their effectiveness*
	TH-LI1-2	**Andrea Ferencz**[1,2], István Láng[3], Eszter Asztalos[3], Imola Nagy[4], Béla Zoltán Debreczeni[5] **15+5'** [1]Department of Surgery, North-Central-Buda Center, New St. John's Hospital and Specialist Clinic, Budapest, Hungary

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[4]Department of Traumatology and Hand Surgery, North-Central-Buda Center, New St. John's Hospital and Specialist Clinic, Budapest, Hungary
[5]DrD Plastic Surgery Studio, Budapest, Hungary

101-Influence of visceral peritoneal desiccation to small bowel microcirculation in animal model

TH-LI1-3 **Imola Nagy[1],** Eszter Asztalos[2], István Láng[2], Andrea Ferencz[3,4], Béla Zoltán Debreczeni[5] **10+3'**

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[4]Doctoral School, Semmelweis University, Budapest, Hungary
[5]DrD Plastic Surgery Studio, Budapest, Hungary

102-Microcirculation changes on nipple and areola complex following different plastic surgical experimental tehniques

TH-LI1-4 **Eszter Asztalos**[1], Istvan Lang[1], Imola Nagy[2], Andrea Ferencz[3,4], Bela Zoltan Debreczeni[5] **10+3'**

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[3]Department of Surgery, North-Central-Buda Center, New St. John's Hospital and Specialist Clinic, Budapest, Hungary
[4]Doctoral School, Semmelweis University, Budapest, Hungary
[5]DrD Plastic Surgery Studio, Budapest, Hungary

103-Effects of botulinum toxin a on microcirculation: laser doppler assessment of blood flow alterations

TH-LI1-5 **István Láng**[1], Eszter Asztalos[1], Imola Nagy[2], Andrea Ferencz[3,4], Béla Zoltán Debreczeni[5] **10+3'**

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[4]Doctoral School, Semmelweis University, Budapest, Hungary
[5]DrD Plastic Surgery Studio, Budapest, Hungary
104-Beneficial effects of anti-inflammatory drugs, negative pressure therapy, autologous fat grafting therapy and hyperbaric oxygen therapy on wound healing

9:30-10:50	TH-LII1	**Unraveling the multiorgan consequences of vascular disease: molecular mechanisms and phenotyping 80'** Chairs: Zsolt Bagi, Nazha Hamdani
	TH-LII1-1	Andrew C. Guilfoyle-Speese[1], Cody L. Bridgewater[1], James D. Mintz[1], David J. R. Fulton[1], **David Stepp[1] 15+5'** [1]Augusta University, United States *106-Increased muscle mass protects against ischemic injury in the lower limb*
	TH-LII1-2	**Etto Eringa[1,2] 15+5'** [1]Department of Physiology, Amsterdam Cardiovascular Sciences Institute, Amsterdam UMC, Amsterdam, the Netherlands [2]Department of Physiology, Cardiovascular Research Institute Maastricht, Maastricht University, Maastricht, the Netherlands *107-Systemic microvascular endothelial dysfunction as a target for diagnosis and treatment of sex-specific cardiac disease*
	TH-LII1-3	**David Fulton15+5'** Augusta University, Augusta, Richmond County, Georgia, United States *108-Role of PBK and metabolic reprogramming in pulmonary vascular remodeling*
	TH-LII1-4	**Paul O'Connor 15+5'** Augusta University, Augusta, Richmond County, Georgia, United States *109- Venous obstruction as the primary cause of tubular injury in ischemic Acute kidney injury.*
10:50-11:20		**Coffee break and industrial exhibition**
11:20-12:00	TH-C3	**ESM, Malphigi Award lecture – Markus Sperandio 40'** Institute of Cardiovascular Physiology and Pathophysiology, LMU Munich, Germany *110- Always along the microvascular path ... and sitting in the neutrophil cockpit* Chair: Henning Morawietz
12:00-12:30	TH-C4	**JVR/ESM Award lecture - Raul Devia-Rodriguez[1,2] 30'** [1]Department of Surgery, Division of Vascular Surgery, University Medical Centre Groningen, University of Groningen, Groningen,

The Netherlands
[2]Surgical Research Laboratory, Department of Surgery, University Medical Centre Groningen, University of Groningen, The Netherlands
111-The influence of oversized plain old balloon angioplasty on endothelial injury in swine arteries
Chair: Nicola Brown

12:30-12:40	**Travel, Oral, Poster Awards and closing**
12:40-13:40	**Lunch**

ESM 2025

MAY 19-22, 2025 • SZEGED, HUNGARY

EUROPEAN SOCIETY FOR MICROCIRCULATION
BIENNIAL MEETING

ORAL ABSTRACTS

EUROPEAN SOCIETY FOR MICROCIRCULATION
Integrating
Vascular Biology & Medicine

1

50 years of microcirculation monitoring and imaging technology

Martin Leahy[1]
[1]University of Galway, Ireland

This paper will mark Mike Stern's 1974 paper first describing the idea of Laser Doppler and early work on video microscopy with the advent of TV video recording equipment. The talk will then move quickly to more modern technologies including Laser Speckle, Diffuse Correlation Spectroscopy, Optical Coherence Tomography Angiography and Photoacoustics, finishing with some comments on the latest advances and the future. Example applications in the skin, brain and eye will be discussed.

2

From brain function to microvessels: functional ultrasound and super-resolution vascular imaging with Iconeus One

Pia Virginia Pelaez[1]
[1]Iconeus, Paris, France.

Functional ultrasound (fUS) imaging is redefining in vivo microvascular studies by enabling high-sensitivity measurements of cerebral blood flow at remarkable spatiotemporal resolution. Built on ultrafast plane-wave sonography, fUS offers real-time imaging at 100 × 100 × 400 µm spatial and 10 ms temporal resolution—allowing researchers to map hemodynamic changes across the whole brain, including in awake, behaving animals.

Complementing fUS, ultrasound localization microscopy (ULM) pushes beyond the diffraction limit by detecting and tracking microbubbles circulating in the vasculature. This super-resolution technique reconstructs detailed maps of vascular density, flow velocity, and microbubble signal amplitude at spatial resolutions down to 5–10 µm—opening new possibilities for studying microcirculation at capillary scale.

This presentation will explore how fUS and ULM together provide a powerful framework for investigating neurovascular function and microvascular architecture. Applications include task-evoked brain activation, dynamic functional connectivity mapping, and in vivo angiography. We will also discuss ongoing advances such as volumetric (3D) imaging and the use of ULM to visualize functional hyperemia and microvascular remodeling with unprecedented detail.

These technologies offer unique opportunities to bridge functional imaging and microcirculation research, enabling multi-scale insight into cerebrovascular physiology, disease models, and therapeutic response. All examples will be illustrated using the Iconeus One system, a dedicated platform for fUS and ULM imaging in preclinical research.

3

Microvascular angina: significance, investigation, and treatment of coronary microvascular dysfunction

Zsolt Piróth
Adult Cardiology Department, Gottsegen National Cardiovascular Center, Budapest, Hungary

Background/Aims: Angina and myocardial ischemia are frequent in patients without obstructive epicardial coronary artery disease. These are caused by microvascular coronary artery disease or coronary vasospasm. The aim of the present paper is to summarize the epidemiology, prognosis, and pathomechanism of microvascular coronary artery disease and vasospastic angina; provide a detailed, technical analysis of the invasive diagnostic modalities, and critically analyze the available therapies.

Introduction: Patients suffering from these conditions have a poor quality of life, and prognosis, frequently needing in-patient care, thereby posing a significant burden on the healthcare system. The pathomechanism of these conditions can be objectively and quantitatively elucidated by invasive coronary thermodilution techniques and provocation tests. Diagnostic criteria of microvascular coronary artery disease and vasospastic angina have been crystallized in recent consensus statements.

Methods and Material: A systematic literature review is provided along with case examples of invasive microvascular testing.

Results: Based on the results of the Cormica trial, a step-by-step invasive microvascular and coronary spasm testing approach is proposed, whereby patients with therapy-refractory angina with non-obstructive coronary artery disease can be classified into microvascular dysfunction, epicardial or microvascular coronary vasospasm, or normal subgroups with a corresponding therapeutic approach. Central to the diagnostic workup is the measurement of bolus thermodilution-based coronary flow reserve, index of microvascular reserve, and calculation of microvascular resistance reserve. Continuous thermodilution (absolute flow measurement) is an emerging but more resource-intensive technique allowing the above measurements to be less operator-dependent and more reproducible.

Conclusion: Systematic application of the diagnostic workup proposed by working groups makes it possible to identify individuals who benefit from medical therapies aimed at the pathomechanism thereby improving the quality of life of these patients.

4

Applications of diffuse optics for detection and characterisation of disease

Hamid Dehghani
School of Computer Science, University of Birmingham, Birmingham, United Kingdom

Background/Aims: Diffuse optical imaging (DOI) of tissue is used to characterize molecular features for the detection, diagnosis, and/or management of diseases. In this work, the underlying principles of Near Infrared (NIR) light propagation in tissue is presented and shown how measures of NIR light transmission through biological tissue can be used to recover patho-physiological information for disease management.

Introduction: The underlying concept of NIR imaging and spectroscopy relies on the unique spectral absorption and scattering properties of biological tissue, which in the NIR wavelength can propagate through thick tissue. Parameter recovery and/or image reconstruction from the measured data is then achieved using computation models to provide functional maps of internal tissue, specifically Hemoglobin, Water, and Lipid. It is through these unique properties that DOI has been developed and utilized in several investigations to demonstrate its contribution for detection, characterization and management of disease, such as cancer, and inflammation.

Methods and Material: Two examples will be presented where spectral NIR data are used to detect and diagnose different types of diseases: (1) Breast Cancer imaging and (2) Rheumatoid Arthritis. These examples utilize differing modes of NIR light imaging, ranging from Frequency modulated to continuous wave imaging modalities. The utilization of multi-modal data are presented to demonstrate how molecular signatures can be recovered through the use of structural maps as obtained from e.g. MRI.

Results: NIR light attenuation is highly affected by the optical properties of the tissue being imaged. Through measurement of NIR light propagation in tissue and model-based optimization algorithms, it is possible to differentiate tissue types through their spectral molecular signatures. Furthermore, as the underlying physiology and morphology of diseased tissue changes with its progression, it is shown that NIR based DOI imaging is able to monitor these changes, non-invasively to provide insight to their development and treatment.

Conclusion: NIR based imaging holds great promise as a non-invasive technique to provide rich, high contrast information about tissue function to allow better understanding of disease detection and management.

5

Laser Speckle Contrast Imaging (LSCI): A Transformative Tool for Decoding Microvascular Dynamics in Health and Disease

Fan Yu
RWD Life Science Co., LTD

Background: Microvascular dysfunction is a critical driver of pathologies such as hypertension, diabetes, stroke, and thrombosis. Traditional imaging methods face limitations in resolution, invasiveness, and real-time monitoring. Laser Speckle Contrast Imaging (LSCI) has emerged as a transformative tool for non-invasive, high-resolution visualization of microcirculatory dynamics.
Objectives: This symposium highlights the application of LSCI in elucidating vascular homeostasis, disease mechanisms, and therapeutic interventions. We demonstrate how LSCI bridges preclinical research and clinical practice through quantitative blood flow analysis, vascular structural/functional assessment, and dynamic monitoring.
Key Content:
Technology Overview:
LSCI Principles: Coherent laser light interacts with moving red blood cells, generating real-time speckle contrast images to quantify perfusion (PU) with high spatiotemporal resolution (up to 100 FPS).
Advantages: Non-contact, dye-free, full-field imaging (2064×1544 resolution), and compatibility with diverse tissues (brain, mesentery, skin).
Research Applications:
Vascular Homeostasis:
- Endothelial/smooth muscle cell interactions, angiogenesis, and inflammatory regulation.
- Epigenetic and nutrient-mediated vascular remodeling.

Disease Models:
- Neurovascular Coupling: Real-time cerebral blood flow (CBF) changes during sensory stimulation in mice.
- Ischemia-Reperfusion Injury: Efficacy of phototherapy in neonatal rat intraventricular hemorrhage.
- Diabetes: Impaired footpad microcirculation linked to neuroimmune dysregulation.
- Thrombosis: NGAL's role in $FeCl_3$-induced carotid occlusion.

Clinical Translation:
- Hypertension: Hemodynamic evaluation of novel vasodilators (e.g., celastrol).
- Dermatology: Monitoring perfusion in acne, dermatitis, and post-RF therapy.
- Surgery: Assessing radial artery cannulation outcomes in hypertensive patients.

Case Studies & Validation:
- Stanford University: Tumor ischemia-reperfusion monitoring and microvascular network design.
- University of Manchester: Post-stroke CBF and electrophysiology correlations.
- Mario Negri Institute: Reproducible cerebral perfusion analysis in subarachnoid hemorrhage models.

Keywords: Laser Speckle Contrast Imaging (LSCI); Microcirculation; Vascular Homeostasis; Real-Time Perfusion; Translational Medicine

6

Regulation of blood flow at the capillary level in health and disease

David Attwell

Department of Neuroscience, Physiology & Pharmacology, and BHF/UK-DRI Centre for Vascular Dementia Research, University College London, London, United Kingdom

It is often assumed that local increases of tissue blood flow are mediated by relaxation of arteriolar smooth muscle but in many tissues, including the brain, heart, kidney and pancreas, capillary control of blood flow by contractile pericytes also occurs. In the brain, most of the adjustable resistance of the intra-cerebral vasculature is located in capillaries. I will demonstrate that neuronal activity mainly increases cerebral blood flow by dilating capillaries via pericytes. In the brain, heart and kidney, ischaemia leads to pericytes constricting, producing a long-lasting decrease of blood flow after ischaemia. Constriction of cerebral capillaries by pericytes also occurs at an early stage of Alzheimer's Disease, when it is expected to amplify the production of amyloid beta. The SARS-CoV-2 virus causing Covid-19 binds to ACE2 on cerebral pericytes and amplifies angiotensin II - evoked pericyte constriction by decreasing ACE2 function. Thus, awareness of the possibility of pericyte-mediated capillary constriction reveals new therapeutic targets to increase blood flow in numerous pathologies.

7

Mechanistic Insights into Coronary Microvascular Disease

Edina Cenko
Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna, Italy

Background/Aims: Coronary microvascular dysfunction (CMD) is a critical contributor to ischemic heart disease, particularly in patients without significant epicardial obstruction. CMD involves endothelial dysfunction, impaired vasomotor regulation, and structural microvascular abnormalities, leading to myocardial ischemia despite normal angiographic findings. Women with acute coronary syndromes (ACS) often exhibit CMD as a primary mechanism, contributing to higher mortality and poorer outcomes compared to men.

Methods and Materials: The vasodilator reserve capacity of the coronary microcirculation is assessed with invasive and non-invasive techniques, including intracoronary acetylcholine testing, myocardial perfusion imaging, coronary flow reserve (CFR), index of microcirculatory resistance (IMR) and more recently the Microvascular resistance reserve (MMR). Clinical and experimental studies assessing microvascular endothelial dysfunction, impaired autoregulation, and endothelial-dependent vasodilation have elucidated some of the CMD's underlying pathophysiology.

Results: CMD is characterized by an imbalance between vasodilatory and vasoconstrictive factors, leading to abnormal microvascular tone and increased myocardial oxygen demand. Patients with CMD exhibit elevated coronary microvascular resistance, impaired endothelial vasodilation, and heightened vasoconstrictor sensitivity. In women with ACS, CMD manifests as reduced TIMI flow post-PCI, even when reperfusion occurs within guideline-recommended timeframes and is associated with increased 30-day mortality. Long-term data suggest that CMD accelerates atherosclerosis and worsens cardiovascular outcomes, even without obstructive disease.

Conclusion: CMD represents a complex interplay between endothelial dysfunction, microvascular remodeling, and autonomic dysregulation, contributing to ischemia beyond traditional CAD paradigms. Restoring endothelial function, improving nitric oxide bioavailability, and modulating microvascular tone are essential therapeutic strategies. A personalized, mechanistically driven approach is crucial to optimizing risk stratification and treatment, particularly in women and high-risk populations.

8

Endothelial derived hyperpolarization has a role coronary microvascular dysfunction

Paul A. Fraser
King's College London, London, United Kingdom

Chest pain is often examined by coronary angiography, but about 40% of female patients have no discernible narrowing of the epicardial arteries, and is now thought to be due to a failure of the small arteries to dilate, hence coronary microvascular dysfunction. Vasoconstriction in small vessels has been shown to be tempered by the passage of Ca2+ from smooth muscle to endothelium (Garland, Sci Signal:2017 10(406)) and dependent on endothelial hyperpolarization via the IKca and SKca channels. The idea presented here is that failure of this mechanism leads to a more severe vasoconstriction.

The Ca2+ dependent potassium channels undergo anterograde and retrograde trafficking (Balut, ChemMedChem 2012 7:1741), disruption of which would diminish EDH, and thus possibly result in CMD. Pre-clinical experiments have indicated that redox balance can disrupt SK trafficking. Porcine coronary arteries treated with homocysteine dilate less well and have reduced SK currents when activated with NS309. The total expression was unchanged, but that on endothelial surface was much reduced (Wang, Atherosclerosis 2015;242:191-8). Furthermore, diabetic coronary arterioles dilated less to vasodilators than matched non-diabetics, and the specific SK currents were much lower, without there being a significant immunoreactive reduction in these channels (Li, J Am Heart Assoc. 2015;4:e002062).

We have carried out some immunofluorescent experiments to test whether redox status would affect SK surface expression in endothelial culture. These which showed that just a brief exposure to H2O2 resulted in a decrease in the surface expression of IKca, without affecting the total quantity.

11

Mitochondrial ROS Influence effect on microvascular ageing

Amanda J LeBlanc[1], Brad Hill[1], Andreas Beyer[2], William Hughes[2], Cristhian Gutierrez[2]
[1]Center for Cardiometabolic Sciences, University of Louisville, United States
[2]The Medical College of Wisconsin, United States

Advanced age is among the leading risk factors for development of coronary artery disease (CAD) but the basic molecular and cellular mechanisms of its pathology remain poorly defined despite decades of research. Most preclinical models fail to recapitulate the complexity of human pathophysiology evident by the fact that numerous clinical studies have failed to show efficacy of free radical scavenging as a “cure”. CAD is traditionally viewed as a large vessel disease but increasing evidence suggest that reduced microvascular endothelial function contributes substantially to development and progression of atherosclerotic vascular disease.

Endothelial dysfunction, particularly in the context of the coronary microcirculation, is the best predictor of major adverse cardiovascular events and uncoupling microvascular function from metabolic demand is thought to contribute to the eventual development of heart failure. One of the hallmarks of heart failure is a shift from mitochondrial fatty acid oxidation (OxPHOS) toward glycolysis, a less efficient means to produce ATP resulting in "metabolic remodeling" of the failing heart. Importantly, patients with CAD exhibit the shift from mitochondrial ATP to glycolysis before a decline in cardiac function itself, suggesting at least a vascular contribution to cardiomyocyte pathology. Further, recent evidence in preclinical models shows that mitochondrial ATP is necessary to maintain physiological NO-mediated vasodilation which is lost in patients with CAD. Understanding the fundamental regulatory mechanism that governs mitochondrial redox environments in the endothelium and the heart itself is a critical step in understanding the pathophysiology of aging-induced decline in cardiac function that leads to heart failure.

A non-canonical role of telomerase reverse transcriptase (TERT) to protect mitochondrial integrity, in part via suppression of DRP1, a key factor that promotes mitochondrial fission, has previously been described and its activation is sufficient to restore physiological vasodilation in the coronary microcirculation of patients with CAD. These findings support the general concept that mitochondrial integrity / function is maintained through a delicate balance between fission and fusion events.

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Differences in vasomotor responses of extra- and intracranial arteries isolated from young and aged pregnant rats

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Background/Aims: Cerebral blood flow (CBF) is regulated by complex mechanisms, which is achieved, in part, by vasomotor responses induced by hemodynamic forces and vasoactive molecules. Pregnancy and age can induce changes in such responses, yet few data are available. We hypothesized that vasomotor function of extracranial, common carotid arteries (CCA) (no space limitation) is different from intracranial, middle cerebral arteries (MCA) arteries (closed intracranial space) and also different in young and aged pregnant rats.

Methods: Young and aged (3-4 and 8-10 months old) pregnant (YP and AP) and non-pregnant, control (YC and AC) female rats were anaesthetized, and their arterial blood pressure (BP) and heart rate (HR) were measured. CCA and MCA were isolated, and their vasomotor responses were measured either with wire myography or with pressure angiography. In MCA pressure-diameter responses and vasomotor reactivity were obtained in response to the thromboxane A_2 analogue U46619. S100B, a calcium-binding protein, was also tested as a biomarker for brain injury. Contractile responses of CCA were obtained to phenylephrine (Phe), angiotensin-II (AngII) and arachidonic acid (AA), and relaxations were measured to acetylcholine (ACh) and S100B. Specific inhibitors of nitric oxide (L-NAME) and prostanoids (indomethacin) were also used.

Results: BP and HR decreased in YP, whereas increased in AP rats (from 80/62mmHg, 365 bpm in AC to 94/74mmHg, 383 bpm in AP, $p<0.05$). In CCA Phe-contraction was higher in the pregnant groups and AngII-induced contraction was reduced in AP compared to YPs. AA-contraction decreased in AP compared to YC. Ach-induced relaxation was impaired in AP compared to non-pregnant, whereas S100B induced relaxation in YP and AC, which was impaired in AP. L-NAME decreased, whereas indomethacin increased relaxations in all groups. In MCA, pressure-and U46619-induced contractions were reduced in YP vs. YC.

Conclusion: Compared to controls, in young and aged pregnant rats: 1) BP and HR increased in AP, 2) vasomotor responsiveness impaired in CCA, 3) pressure-induced basal tone and contraction responses were attenuated in MCA of YP. These findings suggest that vasomotor responses of CCA and MCA are altered in pregnancy, especially in aged rats which may result in disturbed autoregulation of CBF.

Supported by ÚNKP-23-6-SE-7, TKP2021-EGA-37, NKFI OTKA K132596 and Szent-Györgyi Scholarship of National Academy of Scientist Education.

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C3a anaphylatoxin signaling: Bidirectional vasoactive effects in health and vascular disease

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Background/Aims: The complement system has emerged as a critical player in cardiovascular disease (CVD) development, yet its precise molecular mechanisms and signaling pathways remain unclear. This study investigates the vasoactive properties of the C3a anaphylatoxin in the murine thoracic aorta and human coronary arteries, focusing on its modulation in angiotensin II (Ang II)-induced vascular remodeling and ischemic heart disease (IHD).

Methods: Functional vascular responses to C3a were assessed in isolated murine and human vessels using myography. The involvement of the endothelium and adventitia in mediating C3a-induced effects was evaluated, along with the roles of cyclooxygenase-1 (COX1) and thromboxane prostanoid (TP) receptors. C3a receptor (C3aR) expression was examined following Ang II treatment in mice and in coronary arteries from IHD patients. All necessary ethical approvals for animal and human research were obtained before the study.

Results: C3a induced both endothelium-dependent vasodilation and a prominent vasoconstrictive response in murine vessels, the latter being abolished upon adventitia removal. This vasoconstriction was linked to thromboxane A2 (TXA2) release and was absent in COX1- or TP-deficient mice. Ang II administration for two weeks significantly increased C3aR expression and enhanced C3a-induced vasoconstriction, which remained COX1- and TP-dependent. Similarly, human coronary arteries exhibited COX1- and TP-mediated vasoconstriction in response to C3a, with increased C3aR expression and heightened responsiveness in the coronaries of IHD patients.

Conclusion: This study provides the first evidence of TXA_2-mediated signaling of C3aR in murine and human vasculature, demonstrating its upregulation in pathological conditions. These findings suggest that targeting this pathway may hold therapeutic potential for CVDs.

Funding: NKFIH K-135683, K-139230, K-149634, K-149734, K-151053, 2020-1.1.6-JÖVŐ-2021-00010, TKP2021-EGA-25, EFOP-3.6.3-VEKOP-16-2017-00009, 2024-2.1.1-EKÖP-2024-00004, 2024-1.2.3-HU-RIZONT-2024-00059.

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Nucleoside-modified mRNA mediated modulation of organ-specific lymphatic growth and function in preclinical animal models

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Lack or dysfunction of the lymphatic system leads to secondary lymphedema formation that seriously reduces the function of the affected organs and results in the degradation of quality of life. Currently, there is no definitive treatment option for lymphedema. Recently, we utilized nucleoside-modified mRNA encapsulated into lipid nanoparticles (LNPs) encoding Vascular Endothelial Growth Factor C (VEGFC) to stimulate lymphatic growth and function and reduce experimental lymphedema in mouse models. We demonstrated that the administration of a single low dose of VEGFC mRNA-LNPs induced durable, organ-specific lymphatic growth and formation of a functional lymphatic network. Importantly, VEGFC mRNA-LNP treatment reversed experimental lymphedema by restoring lymphatic function without inducing any obvious adverse events.

While mRNA-LNP vaccines have been shown to induce a robust and durable humoral immune response, the underlying molecular and cellular mechanisms involved in this response are still not fully understood. In our current experiments our aim is to investigate the role of lymphatics in nucleoside-modified mRNA-LNP induced organ-specific responses in mouse models. Our results indicate that the lymphatic endothelial cell dependent mechanisms are essential for generating an adequate immune response to mRNA-LNP vaccines, offering valuable insights for optimizing these platforms.

Keywords:
Lymphatic system
Nucleoside-modified mRNA-based platforms
Immune responses

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Development and evaluation of a long-term imiquimod-induced psoriasiform dermatitis model in Balb/c and C57BL/6J mice

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Background/Aims: Aldara cream (5% imiquimod - IMQ)-induced psoriasiform skin inflammation is the most commonly used animal model for studying psoriasis. While the model effectively replicates several aspects of the human condition, it has certain limitations, such as unwanted systemic side effects and an inability to mimic the chronic, relapsing nature of psoriasis. In a previous study, we modified the original IMQ-induced psoriasiform dermatitis protocol using Finn chambers to develop a localized model that eliminates systemic side effects and allows for prolonged imiquimod treatment. In this study, we investigate the long-term application of IMQ in this localized model.

Methods and Material: 20-20 mg Aldara or vaseline were applied simultaneously in two Finn chambers on the dorsal skin of Balb/c or C57BL/6 mice on the first 4 days of the 15-days-long experiment to develop psoriasiform dermatitis. Treatment was continued on every second or third day afterwards in separate groups. Vaseline or corticosteroid were applied on the intermediary days. Skin thickness, blood perfusion, body weight and skin scaling were measured daily. Skin samples were collected on days 5, 10 and 15. Histopathological alterations were assessed on H&E-stained sections, and inflammatory cytokine concentrations were measured with Luminex technology. All procedures were carried out in full accordance with the Ethical Codex of Animal Experiments, under a valid license.

Results: Skin edema increased by 50% or 80% in animals treated every second or third day, respectively. Blood perfusion peaked on day 4 in all treated groups and then gradually decreased until the end of the experiment. Body weight loss was observed after the second Aldara treatment; however, despite further Aldara treatments, the mice’s body weight returned to baseline and continued to increase. Inflammatory cytokine concentrations correlated with the severity of psoriatic symptoms. Topical corticosteroid treatment significantly reduced skin edema starting on day 6, but it had no effect on blood perfusion.

Conclusion: According to our data, application of Aldara cream in Finn chambers for 15 days allows to study chronic skin alterations in psoriasis. Furthermore, this model may be used to investigate the long-term effect of topically applied anti-inflammatory drug candidates in psoriasiform dermatitis.

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Microcirculatory impairment as a precursor to mitochondrial dysfunction in experimental sepsis

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Background: Sepsis leads to a complex imbalance between microcirculatory oxygen delivery and mitochondrial respiration, ultimately driving multiorgan failure (MOF). This study aimed to investigate the relationship between splanchnic microcirculation and hepatic mitochondrial function in a rat model of intraabdominal sepsis, providing insights into their respective roles in the development of MOF.

Methods: Sepsis was induced in male Sprague–Dawley rats by fecal injection ip. The severity of MOF was assessed using the rat organ failure assessment (ROFA) score at various time points after sepsis induction (12, 16, 20, 24, and 28 hours; n=8/group). Microcirculatory parameters, including proportion of perfused vessels (PPV), microvascular flow index (MFI), and heterogeneity index (HI), were evaluated using intravital video microscopy. Hepatic mitochondrial function was analyzed by high-resolution respirometry, measuring oxidative phosphorylation (OxPhos), leak respiration, and the cytochrome c test (CytC%) as an indicator of mitochondrial outer membrane (mtOM) integrity.

Results: The progression of MOF was evident through an increase in ROFA scores, accompanied by microcirculatory dysfunction (a decrease in PPV and MFI and an increase in HI) between 16 and 28 hours. Mitochondrial damage occurred with a 4-hour delay, as indicated by a decrease in OxPhos and elevated CytC%, suggesting mtOM damage. PPV and CytC% showed a significant correlation. Receiver operating characteristic (ROC) analysis identified PPV, CytC%, and lactate levels as strong predictors of MOF. Notably, mitochondrial dysfunction appeared secondary to microvascular failure, suggesting an early role for microcirculatory collapse in the onset of MOF.

Conclusion: Our findings underscore that microcirculatory failure precedes mitochondrial impairment in sepsis, emphasizing the potential of microvascular parameters as early predictors of MOF. The transient decline and rapid recovery of oxidative mitochondrial function suggest the presence of mitochondrial subpopulations that selectively mitigate damage. Future therapeutic strategies should prioritize microcirculatory support to prevent downstream mitochondrial dysfunction and organ failure.

Funding: EFOP-3.6.2-16-2017-00006 and SZTESZAOK-KKASZGYA2025/5S864

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Spatiotemporal adaptation of the cerebrocortical microcirculation to unilateral carotid artery occlusion: the role of NO

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Background/Aims: Investigating the mechanism of cerebral autoregulation has become of notable importance due to the increased incidence of carotid artery stenosis worldwide. We aimed to identify the pattern of cerebrocortical blood flow (CoBF) changes and the role of pial collaterals in the adaptational process after unilateral carotid artery occlusion (CAO). Furthermore, the role of nitric oxide (NO), and especially the NO synthase (NOS) isoforms, were also analyzed.

Methods: Experiments were performed on wild-type (WT), NOS1 KO, NOS3 KO, NOS1/3 DKO (NOS1 and NOS3 double knockout), and WT mice treated with the NO synthase inhibitor L-NAME (all ethical approvals have been obtained). The adaptational capability of cerebrovascular autoregulation was determined by analyzing the regional changes in CoBF using the high temporal and spatial resolution of laser-speckle imaging after unilateral CAO.

Results: Our results show that the Willis circle alone is insufficient to compensate immediately for the loss of one carotid artery, as in the acute phase of the adaptation, a significant CoBF decrease can be seen in the ipsilateral cortex, especially in the temporal region. However, pial collaterals between the frontoparietal and temporal regions attenuate the ischemia of the temporal cortex at the expense of the blood supply of the frontoparietal region. Regarding the role of NOS isoforms, NOS1 KO and NOS3 KO animals show a partially preserved ability for adaptation, whereas an impaired cerebrocortical blood flow adaptation to CAO can be observed in animals lacking both NOS1 and NOS3 isoforms, mainly in the subacute phase. Inhibiting all three NOS isoforms with L-NAME results in severe impairment in CoBF in the acute phase in both hemispheres.

Conclusion: In conclusion, describing the temporal and spatial CoBF changes to unilateral CAO, our experiments provide evidence of the pial collaterals' importance in this adaptational process. Animals deficient in either NOS1 or NOS3 show a preserved adaptational capacity; however, animals lacking both

NOS1 and NOS3 and L-NAME-treated mice cannot compensate efficiently to unilateral CAO, highlighting the significant role of NO in cerebrovascular autoregulation.

Grant support: NRDIO K-125174, K-139230, K-151053, and PD-143327, as well as ÚNKP-22-4-II-SE-17, RRF-2.1.2.-12-2022-0001, TKP2021-EGA-25, R01AG055395, R01AG068295, R01AG070915, R01NS100782, R01CA255840.

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Pathological adaptations of cerebral vasculature in a female Ossabaw swine model of cardiometabolic heart failure with preserved ejection fraction

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Background/Aims: Postmenopausal women pose a higher risk of developing heart failure with preserved ejection fraction (HFpEF) and associated cognitive impairment. Previous data suggests pathological effects of HFpEF on both heart and brain are amplified with a loss of female sex hormones. The aims of this study were to: 1) determine the role of female sex hormones and 2) examine the effects of a novel gene therapy on cerebral arteriole functional and transcriptomic adaptations in a clinically relevant porcine model of HFpEF with metabolic syndrome.

Methods: Female Ossabaw swine were placed on a Western diet at approximately three months of age and at six months of age underwent either aortic-banding (AB, n=4), aortic-banding plus ovariectomy (AB+OVX, n=3), or aortic-banding plus gene therapy (AB+RBD, n=5). Ovariectomy occurred one month prior to aortic-banding. Intravenous delivery of 3 x 10^{14} viral genomes (vg) of a novel cardiac-targeted RSK3/mAKAPβ binding disrupting (RBD) gene therapy occurred immediately following aortic banding. Animals were sacrificed six months post aortic-banding and second order (2A) middle cerebral pial arterioles were isolated for pressure myography and RNA sequencing.

Results: Endothelial-independent sodium nitroprusside-induced dilation (26.9% vs. 83.6%; two-way ANOVA Group Main Effect and Group x Dose Interaction; $p<0.05$) and U46619-induced constriction (38.0% vs. 50.7%; two-way ANOVA Group Main Effect; $p<0.05$) were reduced in AB+OVX compared to AB. Whereas endothelial-dependent bradykinin-induced dilation was improved in AB+RBD compared to AB (77.8% vs. 50.2%; two-way ANOVA Group x Dose interaction; $p<0.05$), and was unaltered by pretreatment with L-NAME (nitric oxide synthase inhibitor) and indomethacin (prostaglandin synthesis inhibitor). RNA seq identified 286 differentially expressed genes (DEGs) in AB+OVX compared to AB, and Ingenuity pathway analysis (IPA) predicted inhibition of brain-derived neurotrophic factor (BDNF) (z-score > -2), a gene regulated by estradiol. RNA seq also identified 26 DEGs in AB+RBD compared to AB, and IPA predicted inhibition of POR, a gene associated with decreased fibrosis and endothelial apoptosis, as well as increased ROS production.

Conclusion: These findings demonstrate: 1) ovariectomy reduced endothelial-independent cerebral vascular function, whereas 2) RBD gene therapy improved endothelial-dependent cerebral vascular function through distinct transcriptomic adaptations in a female swine model of cardiometabolic HFpEF.

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Mitochondrial ATP is necessary for physiological vascular function

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Background/Aims: Arteries and veins are lined by non-proliferating endothelial cells that play a critical role in regulating blood flow. It is thought that endothelial cells rely on ATP generated via glycolysis, rather than mitochondrial oxidative phosphorylation, to fuel each of this energy-demanding process. However, little is known about energy production in endothelial cells within the fully-formed vascular wall. This study was designed to investigate the potential role of mitochondrial ATP production in intact, perfused blood vessels.

Methods and Materials: Acetylcholine-evoked endothelium-dependent vasodilation and calcium signalling in endothelial cells of intact arteries were assessed by fluorescence microscopy. ATP production was assessed in sheets of freshly isolated mesenteric artery endothelial cells. Publicly available single-cell RNA sequencing (scRNA-seq) datasets from patients with vascular disease were analyzed to determine whether mitochondrial function is disrupted in human disease. All necessary ethical approvals for this research have been obtained

Results: Endothelial cell ATP depletion using the mitochondrial ATP synthase inhibitor, oligomycin, inhibited calcium-dependent, nitric oxide-mediated endothelial cell control of vascular tone. Inhibiting mitochondrial ATP synthesis also prevented basal, mechanically-activated, and agonist-evoked IP_3- mediated calcium activity in intact artery endothelial cells. The role of mitochondrial ATP was independent of species, sex, or vascular bed.

Conclusion: We provide evidence that mitochondrial ATP production is crucial for the control of artery diameter. The results suggest that aberrant mitochondrial energy production may underlie endothelial dysfunction. As such, therapeutic intervention to promote endothelial cell mitochondrial energy production may be an effective strategy to combat vascular dysfunction in human diseases.

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Coronary microvascular mechanics and blood flow in type 2 diabetes

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Background/Aims: Previous studies by us and others have documented coronary microvascular disease with reduced tissue and vascular smooth muscle cell (VSMC) stiffness in type 2 diabetes (T2D). The mechanism of reduced coronary microvascular stiffness is unclear, as is its impact on coronary blood flow. As such, we tested the hypotheses that the actin disassembly protein, cofilin, is responsible for reduced coronary VSMC stiffness, and secondarily that reduced VSMC stiffness causes a compensatory increase in coronary blood flow (CBF).

Methods and Material: To test our hypotheses, we utilized a combination of cofilin knockdown experiments, atomic force microscopy, isolated heart preparations, primary VSMCs, and coronary microvascular tissues from normal Db/db and T2D db/db mice. We also developed a novel *in silico* model of coronary blood flow. All animal experiments were approved by the Institutional Animal Care and Use Committee at Nationwide Children's Hospital.

Results: Immunoblot and proteomics demonstrated an increase in cofilin expression in T2D coronary VSMCs together with a concomitant reduction in F-actin expression. Cofilin knockdown in both mouse and human coronary VSMCs using siRNA caused an increase in F/G actin ratio, together with an increase in VSMC stiffness, regardless of diabetic status. Latrunculin B, which reduced coronary VSMC stiffness, caused an increase in CBF in both normal Db/db and T2D db/db isolated mouse hearts, and finding that was corroborated using a novel coronary-cardiac computational model.

Conclusion: Collectively, these data demonstrate that T2D coronary microvascular VSMC stiffness is governed by the actin disassembly protein cofilin. The data further unveil, using both pharmacological modulation of coronary cell stiffness in isolated hearts and a novel computational modeling approach, an inverse relationship between coronary cell stiffness and coronary blood flow in both normal and T2D conditions.

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Damage and potential protection of the coronary microcirculation during myocardial infarction

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Coronary no-reflow is the manifestation of coronary microvascular injury resulting from prolonged and severe myocardial ischemia and reperfusion. No-reflow results from a variety of pathomechanisms, including interstitial, endothelial, and cardiomyocyte edema, impaired vasomotion, leukocyte adhesion and infiltration, stasis of blood elements, and capillary destruction and rupture. No-reflow can occur after prolonged coronary occlusion and reperfusion in a previous virgin coronary territory; therefore, it does not require physical obstruction by atherothrombotic material or the action of soluble factors released from upstream atherosclerotic lesions. Nevertheless, there are typical leukocyte-platelet aggregates and erythrocyte aggregates that obstruct myocardial capillaries with no-reflow. In addition, in a realistic clinical scenario of acute plaque rupture or erosion, there is physical obstruction by atherothrombotic debris and microvascular dysfunction by prothrombotic, proinflammatory, and vasoconstrictor soluble substances. Strictly speaking, cardioprotection refers to the reduction of infarct size resulting from prolonged and severe myocardial ischemia and subsequent reperfusion by mechanical or pharmacological interventions. While microembolization induces patchy microinfarcts, increased expression of TNF not only causes progressive contractile dysfunction but is also cardioprotective. In anesthetized pigs, TNF expression was increased and infarct size was reduced hours after coronary microembolization; this cardioprotection was abolished by TNF antibodies. While coronary microembolization per se appears to be cardioprotective under certain circumstances, it does not interfere with the cardioprotection produced by ischemic preconditioning or postconditioning. RBCs are involved in the synthesis and transport of vasoactive nitric oxide. RBC nitric oxide synthase (RBC NOS) is involved in RBC deformability through s-nitrosylation of RBC cytoskeletal proteins, and RBCs contribute to the regulation of circulating NO metabolites and blood pressure. During myocardial ischemia/reperfusion, RBC NOS bioactivity is cardioprotective, and there is preliminary evidence that RBCs also contribute to cardioprotection by reducing coronary no-reflow.

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Autoimmunity and coronary microvascular dysfunction: new actors in acute myocardial infarction?

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Acute myocardial infarction (AMI) is traditionally linked to obstructive coronary artery disease and plaque rupture. However, increasing evidence highlights coronary microvascular dysfunction (CMD) and autoimmunity as significant, yet underrecognized, contributors to myocardial injury.

CMD involves abnormalities in the coronary microcirculation, independent of epicardial stenosis. It leads to impaired myocardial perfusion and can cause ischemia or infarction, especially in patients without obstructive disease (MINOCA). CMD mechanisms include endothelial dysfunction, poor vasodilation, and microvascular spasm, often worsened by systemic inflammation and immune-mediated vascular damage.

A notable clinical feature of CMD is the no-reflow phenomenon—where, despite successful reopening of an epicardial artery during percutaneous coronary intervention (PCI), myocardial perfusion remains inadequate. No-reflow is linked to larger infarcts, reduced ventricular function, and worse outcomes. Immune processes such as leukocyte plugging, complement activation, and cytokine release play key roles in this condition.

Autoimmunity, marked by autoreactive cells and autoantibodies, is increasingly recognized in cardiovascular pathology. Conditions like lupus and rheumatoid arthritis frequently exhibit CMD, correlating with higher cardiovascular risk. Even in the absence of overt autoimmune disease, subclinical autoimmunity may impair the microvasculature via chronic inflammation and immune system activation.

This presentation reviews evidence connecting CMD and autoimmunity to AMI, drawing from imaging, biomarkers, and pathology studies. It also discusses diagnostic tools such as coronary flow reserve testing, cardiac MRI, and immune profiling, along with emerging immunomodulatory treatments targeting microvascular injury.

Recognizing CMD and autoimmunity as contributors to AMI shifts the focus beyond epicardial disease, advocating for more personalized approaches to diagnosis, risk assessment, and therapy.

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Empagliflozin prevents oscillatory shear stress-induced oxidative stress in human coronary artery endothelial cells via inhibition of NHE1/NCX

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Background: Sodium glucose co-transporter 2 inhibitor (SGLT2i) EMPA is an anti-diabetic drug with profound cardioprotective effect in heart failure patients regardless of diabetes mellitus. Endothelial cells (ECs) in vivo are constantly exposed to multiple mechanical forces such as cyclic stretch and shear stress. Our previous study suggested that EMPA directly exerts anti-oxidative effects in human ECs subjected to cyclic stretch through the inhibition of sodium-hydrogen exchanger 1 (NHE1)/sodium-calcium exchanger (NCX) and decreased intracellular calcium (Ca^{2+}). ECs form the inner layer of blood vessels and primarily sense shear stress resulting from blood flow. Oscillatory shear stress (OSS) induces endothelial dysfunction and increases reactive oxygen species (ROS) production. In addition, the mechanically activated Piezo-1 channel induces Ca^{2+} influx and ROS signaling in ECs. Here, we hypothesized that EMPA ameliorates ROS generation in ECs undergoing OSS through modulating NHE1, NCX and Piezo-1.

Methods: Human coronary artery endothelial cells (HCAECs) were pre-incubated for 2 h with either vehicle, EMPA, or NHE1 inhibitor Cariporide, or NCX inhibitor ORM-10962 followed by exposure to laminar shear stress (LSS, as control) or OSS. Piezo-1 was silenced using siRNA. ROS generation was detected by live cell imaging after 6 h. At 24 h, NHE1 and Piezo-1 protein expression was detected with Infra-red western blot. Additionally, freshly isolated mouse aortas were incubated with either vehicle or EMPA for 4 h. Superoxide dismutase type 1 (SOD1) was analyzed by en-face immunofluorescence staining to observe ROS signaling in the endothelium of the thoracic (subjected to LSS) and the aortic arch (subjected to OSS).

Results: *In vivo*, SOD1 expression was significantly lower in the aortic arch compared to the thoracic aorta ($P<0.05$). EMPA treatment increased SOD1 expression in the aortic arch. *In vitro*, OSS markedly increased ROS production in HCAECs compared to LSS ($P<0.05$), whereas EMPA reduced ROS generation under OSS ($P<0.05$). NHE1 and Piezo-1 expression were unaffected by OSS ($P>0.05$). Cariporide, ORM-10962, and knockdown of Piezo-1 each reduced OSS-induced ROS generation in HCAECs ($P<0.05$). However, combining EMPA with either cariporide or ORM-10962 did not enhance the anti-oxidative effect of EMPA, whereas enhancement was still observed in Piezo-1 knockdown HCAECs (OSS+SCR vs OSS+KD+EMPA, $P>0.05$).

Conclusion: EMPA reduced OSS-induced oxidative stress in HCAECs probably through inhibition of the NHE1/NCX axis rather than Piezo-1.

Key words: Sodium glucose co-transporter 2 inhibitor, endothelial cells, sodium-hydrogen exchanger 1, sodium-calcium exchanger, reactive oxygen species

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Mental stress and coronary microvascular dysfunction: a new brain-heart axis?

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Growing evidence highlights the critical role of mental stress in cardiovascular disease. Both the activation of specific brain regions—such as the amygdala—and the presence of mental health conditions like depression are linked to an increased risk of atherosclerotic cardiovascular disease. Intriguingly, mental stress also shows a strong association with coronary microvascular dysfunction.

In this presentation, we will explore the emerging evidence on:

1. The relationship between mental stress, arterial inflammation and endothelial dysfunction
2. The association between mental stress and coronary microvascular dysfunction
3. The role of mental health in coronary syndromes with non-obstructive coronary arteries (e.g., MINOCA and INOCA)
4. The potential of various stress reduction strategies to improve microvascular function

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Amyloid-β effects on the blood-brain barrier: contribution to Alzheimer's disease

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Background/Aims: Around 80% of Alzheimer's disease (AD) cases show cerebral amyloid angiopathy (CAA) with amyloid-β (Aβ) deposition around blood vessels. Although blood-brain barrier (BBB) impairment in AD has been reported, detailed information on changes in cell types of the neuro-vascular unit (NVU) is missing.

Methods: To investigate CAA-related effects on the BBB and on pathology progression, we made use of the Thy1-SwDI mouse model, showing onset of CAA plaques and AD symptoms by 3 months of age and performed RNA-Seq on FACS-sorted cells of the NVU.

Results: We show BBB deficits in AD mice by tracer experiments with 4KD-FITC dextran. RNA-Seq of NVU cells of AD mice during ageing (6 vs. 18 month) identified a significant transcriptomal change at the NVU during healthy ageing, which was partially seen in young AD mice. Amon the regulated pathways, a downregulation of Wnt/β-catening in ECs was validated at the protein level in both mouse and human AD samples. Strykingly, Dkk2, a secreted Wnt inhibitor, was strongly up-regulated in microglia of AD samples. DKK2 immunoreactivity was accentuated around neuritic plaques together with microglia of autopsy brain samples from AD patients. In *in vitro* cultured murine brain microvascular ECs (MBMECs), microglia-derived Dkk2 reduced resistance in TEER measurements indicative of reduced barrier properties upon Wnt/β-catenin pathway inhibition.

Conclusion: Our data clearly show that cell type-specific analysis is key to identify regulatory networks in AD and other pathologies at the NVU. Our data suggests that microglia-derived Dkk2 modulates Wnt/β-catenin and BBB properties in AD. We hypothesise that the Wnt/β-catenin pathway at the BBB might be a potential therapeutic target.

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Engineering microfluidic chip models of the blood-brain barrier

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Novel lab-on-a-chip and organ-on-a-chip cell culture models are essential to investigate cerebral drug delivery, pathology and protection of tissues in disease states. Microfluidic chip devices allow complex and physiological modelling of the blood-brain barrier (BBB) and enable the co-culture of multiple human cell types, also of induced pluripotent stem cell (iPSC) origin. Since the 2010's an interdisciplinary area of research has risen: the development of lab-on-a-chip technology incorporating cell culture BBB models (Deli et al, 2024). Organ-on-a-chip devices are employed to create dynamic, microfluidics-based BBB models, such as our versatile microelectronic chip created in 2016. This platform offers a more physiologically relevant system compared to static models and is able to mimic the complex microenvironment of the BBB, simultaneous resistance and permeability measurement and the co-culture of three primary cell types (Walter et al, 2016). We were also able to introduce dynamic fluid flow in the system creating shear stress, which proved to be crucial for establishing and maintaining BBB functions and integrity (Santa-Maria et al, 2021).

To enhance our understanding of BBB physiology and pathology, and to provide a valuable platform for drug development and disease modeling more complex models are needed. The latest trend in the pharmaceutical drug testing is the use of human brain organoids derived from iPSCs. Recently we have developed an innovative, one-of-a-kind version of an organ-on-a-chip device. Using a BBB co-culture model of hematopoietic stem cell-derived human brain endothelial cells and brain pericytes (Cecchelli et al, 2014), we established a robust barrier system in our chip device to which we integrated human midbrain organoids (Kocsis et al., 2025). This approach is unique in both the BBB model and the lab-on-a-chip fields, as it not only enables the study of the BBB but also its interactions with brain organoids. This novel system allows to analyze molecule permeability across the brain endothelial monolayer while tracking the entry of agents into the organoid (Mészáros et al. 2025). We confirm that all necessary ethical approval for our research has been obtained. With the help of this system we can create personalized models of central nervous system diseases to investigate the effects of various pathological factors and protective compounds on both brain endothelial cells and brain organoids.

Funding: The project was supported by the National Research, Development and Innovation Office (NKFIH), NNE-129617 & OTKA-K 143766 (to M.A.D.), the Secretariat of the Hungarian Research Network (former ELKH, SA-111/2021 to F.R.W.), and by the University Research Scholarship Programme (EKÖP-24-2-SZTE-449 to A.E.K.), which is a sholarship of the Ministry of Culture and Innovation funded by the NKFIH.

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Senolysis potentiates endothelial progenitor cell adhesion to and integration into the brain vasculature

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Background/Aims: One of the most severe consequences of ageing is cognitive decline, which is associated with the dysfunction of brain microvasculature. Thus, repairing brain vasculature could result in healthier brain function.

Methods: To better understand the potential beneficial effect of endothelial progenitor cells (EPCs) in vascular repair, we studied the adhesion and integration of EPCs using the early embryonic mouse aorta–gonad–mesonephros – MAgEC 10.5 endothelial cell line. The EPC interaction with brain microvasculature was monitored ex vivo and in vivo using epifluorescence, laser confocal and two-photon microscopy in healthy young and old animals. The effects of senolysis, EPC activation and ischaemia (two-vessel occlusion model) were analysed in BALB/c and FVB/Ant:TgCAG-yfp_sb #27 mice.

Results: MAgEC 10.5 cells rapidly adhered to brain microvasculature and some differentiated into mature endothelial cells (ECs). MAgEC 10.5-derived endothelial cells integrated into microvessels, established tight junctions and co-formed vessel lumens with pre-existing ECs within five days. Adhesion and integration were much less pronounced in aged mice, but were increased by depleting senescent cells using abt-263 or a combination of dasatinib plus quercetin. Furthermore, we demonstrated that MAgEC 10.5 interaction with brain vessels could be upregulated by ischaemic conditions and the inflammatory mediator TNFα. Thus, even though progenitor cell therapy using MAgEC 10.5 was rendered less effective by aging, it could be improved by modulating EPC-vasculature interactions through senolytics or by activating EPCs before treatment.

Conclusion: Combining progenitor cell therapy with senolytic therapy and the prior activation of EPCs are promising for improving EPC adhesion to and integration into the cerebral vasculature and could help rejuvenate the ageing brain.

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Effect of co-exposure to high-fat diet and low or moderate doses of ionizing radiation on the cerebral microcirculation

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Background/Aims: Epidemiological studies carried out on cohorts of nuclear workers suggest a relationship between exposure to low and moderate doses of ionizing radiation (IR) and the risk of cerebrovascular diseases and dementia (Gillies et al, 2017; Laurent et al, 2023). To date, experimental studies on the risk of vascular disease at low-doses range have mainly focused on inflammatory diseases of large vessels such as atherosclerosis or aneurysms and have shown no deleterious effect. Microvascular alterations, however, which are strongly associated with cerebrovascular pathologies and their morbidity, have mainly only been studied at high dose ranges. Furthermore, established risk factors such as obesity and dyslipidemia increase the incidence of microvascular diseases. This study aims at identifying the response of cerebral microcirculation to acute exposure to low-to-moderate doses of IR, associated or not with a lipid-rich diet.

Methods and Material: 8-week-old male C57BL/6J mice fed either with a high-fat diet or a normal diet, were acutely exposed to total-body irradiation by an external source of gamma ionizing radiation at doses of 300 mGy and 1 Gy. Sacrifice and analysis were conducted at 4 time-points after irradiation, spanning both short term (24 hours and 15 days) and long term (3 months and 6 months) periods. Microvascular density in the hippocampus was assessed by immunostaining of vascular markers in brain slices. Expressions of genes related to processes like endothelial dysfunction, hypoxia and angiogenesis were evaluated by RT-PCR.

Results: We show that at 3 months post-irradiation exposure to IR alone, at any dose, has no impact on the density of cerebral vascularization, nor does the lipid-rich diet. The same applies to the exposure to the lipid-rich diet combined to the 300 mGy radiation dose. However, co-exposure to a lipid-rich diet and to the 1 Gy radiation dose induced an increase in hypoxia and angiogenesis processes in the short term, and a rise in vascular density at 3 months post-exposure, compared with control groups.

Conclusion: Co-exposure to high-fat diet and moderate dose (1Gy) of gamma IR increase vascular density in the brain hippocampus, a process probably following hypoxia-like induced angiogenesis.

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High salt diet increases oxidative stress and alters vasodilation in human microcirculation

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Background/Aims: High salt (HS) intake is a well-established risk factor for arterial hypertension, but it may also adversely affect vascular endothelial function, even in the absence of changes in blood pressure. This is an overview of several studies of our research group investigating the short-term effects of a high-salt diet on oxidative stress and its role in endothelial dysfunction, specifically in the microvasculature.

Methods and Material: Studies were designed as a non-randomized, controlled interventional experiments involving young healthy participants. Subjects maintained a 7-day low-salt diet (<3.5g salt/day) followed by a 7-day HS diet (∼14g salt/day) or a HS diet with vitamin C and E (∼14g salt + 1000mg vitamin C + 800IU vitamin E/day). Endothelium-dependent and -independent skin microvascular dilation was assessed by measurement of post occlusive reactive hyperemia, and acetylcholine- and sodium nitroprusside-induced dilation using laser Doppler flowmetry. Several biomarkers of oxidative stress (reactive thiobarbituric acid substances, 8-iso prostaglandin F2α, intracellular production of reactive oxygen species) and antioxidant defense (plasma iron-reducing capacity and antioxidant enzymes protein concentrations) were measured before and after the dietary protocols.

Results: The first set of our experiments demonstrated that 7-day HS diet impairs peripheral microvascular reactivity by affecting its endothelium-dependent, but not endothelium-independent vasodilation. Changes were independent of blood pressure, body composition changes or fluid retention. The HS diet led to a decrease in antioxidant capacity and an increase in oxidative stress independent of blood pressure. The second set of experiments showed that enhanced antioxidant defense by administration of vitamin C and vitamin E during HS loading reversed changes in oxidative status and prevented impairment of peripheral microvascular endothelial function that was observed with HS diet.

Conclusion: These studies provided the pivotal results on the role of oxidative balance in maintaining normal vascular endothelial function during HS loading, independently of blood pressure values.

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The effect of acute hyperglycemia on skin microvascular reactivity evaluated by an integrated approach

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Background/Aims: Both, glucose and insulin have been shown to affect endothelial microvascular function and the sympathetic autonomic nervous system. How their effects integrate at the microcirculation level remains to be elucidated as *in vivo* human studies show inconsistent results. We aimed to investigate the effect of acute hyperglycemia on cutaneous microvascular reactivity in healthy young volunteers while simultaneously assessing glucose and insulin concentration.

Methods: Acute hyperglycemia was provoked by a standard oral glucose tolerance test (OGTT) and the control protocol was repeated by drinking water. Laser-Doppler flux (LDF), arterial blood pressure, and electrocardiogram (ECG) were simultaneously recorded. Vascular reactivity was assessed by various provocations: post-occlusive reactive hyperemia (PORH) induction, and iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP), respectively, before and 40 and 120 minutes after OGTT and water, respectively. Heart rate variability (HRV) was assessed as a measure of autonomic nervous system (re)activity and wavelet analysis (WA) was performed on LDF-acquired signals to estimate the physiological mechanisms controlling vascular reactivity. Participants had their glucose and plasma insulin measured at various time points according to the testing protocol. All necessary ethical approvals have been obtained.

Results: While robust measures did not show any significant changes, a more delicate analysis using WA and HRV unrevealed subtle changes that might be the consequence of acute hyperglycemia and/or hyperinsulinemia. Our main finding was a significant interaction between time and intervention (glucose/water loading) in the endothelium-dependent, nitric oxide (NO)-independent (NOi; $p=0.014$), and myogenic ($p=0.029$) bands evaluated from the SNP response (two-way repeated measures ANOVA), whereas no effect was shown for the endothelium NO-dependent component. Significant correlations between some PORH and all HRV parameters and the glucose concentration increase after OGTT were found implying diminished vascular reactivity in healthy subjects exhibiting lower glucose sensitivity/tolerance.

Conclusion: Acute hyperglycemia affects microcirculation time-dependently and transiently, likely through modulation of endo NOi signaling and vascular smooth muscle responsiveness indicating that glucose's effects may be selective rather than indicative of a global impairment. Moreover, the glucose tolerance might determine vascular reactivity shortly after OGTT stressing the need to develop stronger tools/markers for a timely detection of predisposed individuals.

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Microvascular function in children with hypertension

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Background/Aims: Hypertension has become a significant global health issue, even in children. While it is well-established that hypertension in adults leads to impaired vascular function, particularly in relation to the endothelium, there is limited research on how primary hypertension affects vascular function in children. This paper aims to consolidate the data we obtained in our investigation on the impact of primary hypertension on vascular function in the pediatric population

Methods and Material: A total of 52 children participated in this study: 26 children with essential arterial hypertension (HT) and 26 normotensive children (NT) (both sexes, age ranged 9-17 years). Their anthropometric, hemodynamics, and biochemistry parameters are determined. Further, peripheral microvascular responses were measured using laser Doppler flowmetry (LDF), in response to vascular occlusion (post-occlusive reactive hyperemia (PORH)), local skin heating (local thermal hyperemia (LTH)), and iontophoretically applied acetylcholine (AChID) and sodium nitroprusside (SNPID). The study was approved by the Ethical Committee of the University Hospital Osijek, Osijek, Croatia (R1/6414/2021) and the Ethical Committee of the Faculty of Medicine Osijek (#2158-61-07-21-06). The present study is a part of the registered clinical trial database at ClinicalTrials.gov, (ID NCT05109013).

Results: Body mass index (BMI) was significantly higher in the HT group compared to the NT group, while waist-to-hip ratio (WHR) was similar between the two groups. Children with primary hypertension had significantly higher systolic (SBP), diastolic (DBP), and mean (MAP) arterial blood pressure compared to normotensive children, with no difference observed in heart rate. There was no difference between groups in full blood counts, serum electrolytes, and renal function markers. In contrast, children from the HT group had higher cholesterol values (total, HDL, and LDL cholesterol). Still, the values were within the reference range. In children with hypertension, PORH, AChID, and LTH responses were significantly impaired compared to normotensive children. However, SNPID did not show a significant difference between the groups.

Conclusion: Primary hypertension in childhood is linked to impaired endothelial function and reduced endothelial-dependent responses to physiological stimuli, independently of body weight.

Funded by the Faculty of Medicine Osijek Institutional Research Projects IP-09-MEFOS-2021 (PI Ivana Jukić) and IP-17-MEFOS-2022 (PI Ivana Jukić).

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Intermittent hyperbaric oxygenation restores the mechanisms of vasodilation in rat animal model

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Background/Aims: The positive effects of hyperbaric oxygenation (HBO_2) on tissue perfusion are well established, however, the underlying mechanisms through which HBO_2 enhances tissue oxygenation remain insufficiently understood**.** This study investigated the effects of acute and intermittent hyperbaric oxygenation (HBO_2) on endothelial function, oxidative stress, and molecular mechanisms in blood vessels, focusing on endothelium-dependent vasorelaxation, superoxide production, and antioxidative enzyme activity in the aorta and middle cerebral artery of Sprague-Dawley (SD) rats.

Methods: Male SD rats were randomized into four groups: CTRL (untreated), A-HBO_2 (single HBO2 session, 100% O_2 at 2.0 bars for 120 min), 24H-HBO_2 (examined 24h after a single session), and 4D-HBO_2 (four consecutive daily sessions). Vasorelaxation in response to acetylcholine (AChIR) and hypoxia (HIR) was assessed in isolated aortic rings with/without NOS inhibitor L-NAME and superoxide scavenger TEMPOL. The mRNA expressions of vascular and antioxidative enzymes were measured by RTqPCR. Serum oxidative stress markers and enzyme activities were determined spectrophotometrically, and vascular superoxide production was measured using DHE fluorescence. The role of arachidonic acid (AA) metabolites in vascular responses was determined via LC-MS/MS. Flow-induced dilation (FID) was assessed in middle cerebral arteries.

Results: A-HBO_2 impaired AChIR and HIR, restored by TEMPOL. Increased serum oxidative stress and superoxide production were observed in A-HBO_2. iNOS expression was reduced in A-HBO_2 and 24H-HBO_2, while elevated in 4D-HBO_2. Catalase and glutathione peroxidase expression and activity were increased in 4D-HBO_2. HIR was mediated by prostanoids and epoxygenases, with increased HIF-1α expression in 24H-HBO_2 and 4D-HBO_2. HBO_2 influenced COX-1, COX-2, and CYP2c11 expression. AA metabolite levels, including 14(15)-DiHET and 8(9)-EET, were elevated in A-HBO_2 and 24H-HBO_2. FID was reduced in A-HBO_2 due to oxidative stress and NO depletion but was restored by superoxide scavenging. 4D-HBO_2 activated antioxidative mechanisms and shifted FID from NO-dependent to epoxygenase-mediated, involving TRPV4 and potassium channels.

Conclusion: Acute HBO_2 transiently impairs vasorelaxation due to oxidative stress, with recovery 24h post-exposure. 4D-HBO_2 enhances antioxidative defenses and shifts FID from NO-dependent to epoxygenase-mediated responses. These findings highlight HBO_2's complex effects on vascular function and oxidative stress regulation.

Funded by Faculty of Medicine Osijek institutional grants: VIF07-2018-MEFOS, IP2-2019-MEFOS, IP8-2020-MEFOS, IP5-2021-MEFOS, IP25-2024-MEFOS, and IP7-2025-MEFOS.

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Microvascular functions in postischemia neovascularisation and type 2 diabetes

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Insulin resistance is a central pathomechanism of obesity-induced type 2 diabetes. Insulin's actions are not restricted to metabolic cells but also involve the vascular endothelium where insulin increases blood flow and delivery of insulin as well as nutrients. The importance of endothelial insulin signaling for insulin resistance and its relevance as a potential therapeutic target are, however, unclear. Here we show that adrenomedullin, whose plasma levels are increased in obese humans and mice, inhibited insulin signaling in human endothelial cells through protein kinase A (PKA)-mediated phosphorylation and activation of protein tyrosine phosphatase 1B (PTP1B) resulting in dephosphorylation of the endothelial insulin receptor. The adrenomedullin binding protein complement factor H whose levels are also elevated in obese humans and mice promotes adrenomedullin effects. In obese mice lacking the endothelial adrenomedullin receptor or the G-protein G_s, insulin-induced endothelial NO-synthase activation and skeletal muscle perfusion were increased. Treatment of lean mice with adrenomedullin mimicked the effect of obesity and induced systemic insulin resistance involving the endothelial adrenomedullin receptor. Endothelium-specific loss of the adrenomedullin receptor and G_s or treatment with an adrenomedullin receptor antagonist improved obesity-induced insulin resistance. This identifies a mechanism underlying obesity-induced endothelial and systemic insulin resistance and suggest approaches to prevent and treat type 2 diabetes.

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Coronary microvascular function in health & ischemic heart disease

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The role of perturbations in coronary microvascular structure and function in ischemic heart disease is becoming increasingly recognized, but the microvascular mechanisms that control coronary blood flow and the underlying causes and mechanisms of coronary microvascular dysfunction, remain incompletely understood. In this presentation the physiological regulation of coronary blood flow will be briefly discussed followed by discussion of translational studies of coronary microvascular dysfunction in models of systolic dysfunction (post-infarct remodelling) and diastolic dysfunction (co-morbidities, including metabolic perturbations and chronic kidney disease). The results from these studies indicate that coronary microvascular dysfunction is a key feature of various pathophysiological states, indicating that the coronary microcirculation represents an important therapeutic target for the treatment of cardiovascular disease.

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Coronary microvascular dysfunction in metabolic disease

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Heart failure with preserved ejection fraction (HFpEF) is increasingly recognized as a syndrome driven, in part, by coronary microvascular dysfunction and rarefaction, which impair myocardial perfusion and contribute to left ventricular (LV) diastolic dysfunction. We hypothesize that targeted modulation of vascular signaling pathways can restore microvascular integrity and improve cardiac performance in HFpEF. Our studies demonstrate that pharmacological inhibition of adenosine kinase (ADK) and phosphodiesterase 9A (PDE9A) enhances coronary microvascular vasodilator function and ameliorates LV diastolic abnormalities, supporting a mechanistic link between nucleotide signaling and vascular health in HFpEF. Furthermore, large-scale proteomic analyses of myocardial and vascular tissues from HFpEF models reveal dysregulation in metabolic, inflammatory, and cytoskeletal pathways that may underlie microvascular disease. We propose that these proteomic signatures can serve both as mechanistic insights and as targets for therapeutic intervention. Based on these findings, we hypothesize that spatially and temporally controlled activation of endogenous vasoprotective mechanisms—such as purinergic and cyclic nucleotide signaling—can yield sustained improvements in coronary microvascular function and overall HFpEF outcomes. This approach opens new avenues for precision therapies aimed at restoring vascular homeostasis in HFpEF.

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Microvessels, macro impact: the role of capillaries in healthy aging

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Background and aims: As life expectancy rises, age-related diseases are becoming increasingly prevalent, largely due to declining cellular and tissue repair mechanisms. While traditionally studied in isolation, growing evidence suggests shared systemic underlying mechanisms. The vasculature, a key regulator of organ homeostasis and repair, is emerging as a central driver of aging-related dysfunction, positioning vascular impairment as a fundamental biological mechanism underlying multiple age-associated diseases.

Material and Methods: Using a transgenic mouse platform for vascular modulation, we investigate how VEGF signaling impacts microvascular function, aging-associated pathologies, and healthspan.

Results: Counteracting age-related microvascular rarefaction via VEGF signaling induces comprehensive geroprotection, preserving organ function and delaying the onset of multiple aging-related diseases. Conversely, premature blockade of VEGF signaling accelerates the onset of age-related conditions, including Heart Failure with Preserved Ejection Fraction (HFpEF), demonstrating a causal role for impaired VEGF signaling in this complex, aging-associated syndrome.

Our findings further reveal that microvascular rarefaction is closely linked to alterations in the alternative RNA splicing machinery, with VEGF insufficiency driving the production of aberrant splicing variants, such as the soluble decoy receptor sFlt1, a known contributor to vascular dysfunction. Restoring microvascular function not only improves tissue perfusion but also reverses these splicing alterations, suggesting a mechanistic link between vascular health and RNA processing dynamics. While hypoxia-driven and age-related RNA splicing changes have been studied independently, our findings propose a unifying mechanism that connects the two. To further investigate these mechanisms, we employ patient-derived organoids, which retain their biological age in culture, providing a robust in vitro platform to test anti-aging interventions.

Conclusion: Our findings support **a vascular theory of aging**, suggesting that age-related microvascular rarefaction acts as an upstream driver of systemic aging. Targeting vascular function may offer a promising therapeutic strategy for mitigating age-related diseases and promoting healthier aging

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Inflammation and oxidative stress in endothelial-cardiomyocyte interactions: insights into heart failure with preserved ejection fraction

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Heart failure with preserved ejection fraction (HFpEF) is a multifaceted syndrome characterized by systemic inflammation, oxidative stress, and comorbidities such as diabetes, obesity, and atrial fibrillation. Emerging evidence highlights the central role of endothelial dysfunction and its maladaptive interaction with cardiomyocytes in promoting myocardial stiffness and diastolic dysfunction. Chronic low-grade inflammation and oxidative injury activate pathological signaling pathways that drive adverse protein modifications such as phosphorylation, nitrosylation, and oxidation of key structural and regulatory proteins. These molecular alterations impair cardiomyocyte relaxation, promote fibrosis, and alter endothelial barrier function. I will explore how inflammation-mediated signaling and post-translational modifications at the endothelial-cardiomyocyte interface contribute to HFpEF pathogenesis, with a special focus on the exacerbating impact of metabolic comorbidities. Understanding these mechanisms offers critical insights for developing targeted therapies aimed at restoring cellular homeostasis and improving outcomes in HFpEF patients.

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Age-related vascular cognitive impairment: role of endothelial senescence

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Age-related phenotypic changes in cerebromicrovascular endothelial cells contribute to the dysregulation of cerebral blood flow and disruption of the blood-brain barrier, thereby promoting the development of vascular cognitive impairment (VCI). In recent years, there has been growing recognition of endothelial cell senescence as a potential underlying mechanism driving microvascular abnormalities. This realization has paved the way for exploring the potential of senolytic drugs in preclinical research to improve cererbromicrovascular function and structure and positively impact cognition.

In order to identify senescent endothelial cells in preclinical mouse model of aging, we used single cell RNA sequencing to meticulously examine cells derived from enriched fractions of cerebromicrovascular endothelial cells and other components of the neurovascular unit. We characterized and delineated 13 distinct transcriptomic cell types. Our investigation revealed a notable elevation in the proportion of senescent endothelial cells within the cerebral microcirculation of aged mice. This phenomenon was further validated using spatial transcriptomics, which confirmed the heightened presence of senescent endothelial cells within key regions such as the hippocampus and cortex of the aged mouse brain.

To investigate the impact of senescence on the cerebromicrovascular endothelial phenotype and function, including NVC and maintenance of BBB and microcirculatory network architecture we tested the efficacy of a clinically relevant senolytic treatment regimen, and administered the BCL-2/BCL-xL inhibitor senolytic drug ABT263/Navitoclax to aged mice. Encouragingly, our findings indicate that Navitoclax intervention leads to an enhancement in neurovascular coupling and decreased blood brain barrier permeability among aged mice, which in turn correlates with significant improvements in cognitive performance. These findings collectively underscore the potential therapeutic utility of senolytic drugs across various age-related cerebrovascular conditions, as underscored by our preclinical investigations. By shedding light on the role of endothelial cell senescence and its modulation, our results open the avenue to the therapeutic exploitation of senolytic drugs in multiple age-related cerebrovascular pathologies in preclinical studies.

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Vascular function and brain clearance measurements in a mouse model of cerebral amyloid angiopathy

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Background/Aims: Cerebral amyloid angiopathy (CAA) is a neurovascular disease that leads to intracerebral hemorrhage and cognitive decline. Accumulation of amyloid-β peptides in the vessel wall is the pathological hallmark of the disease, suggested to be the result of a downward spiral between vascular dysfunction and impaired brain clearance. To test this hypothesis, we measured vascular function and brain clearance in the APP23 mouse model of CAA, using optical imaging and MRI.

Methods and Material: For the optical imaging experiments, a cranial window surgery was performed in 24 mice. In 9 of these mice (wildtype only), a silicon port was included above the visual cortex to allow for intra-cortical tracer injections. Vessel diameter changes in response to visual stimulation (flashing checkerboard, 5 s on/15 s off) or spontaneous vessel diameter changes (vasomotion) were used as estimates of vascular function. Signal decrease of the injected tracer (50 nL of 40 kDa fluorescent dextran) over time was used as an estimate of brain clearance.

For the MRI experiments, APP23 mice at the ages of 12, 18 and 24 months without cranial windows (n=40) were imaged freely-breathing under isoflurane anesthesia with non-invasive perfusion-weighted imaging using Arterial Spin Labeling (ASL)-MRI. Here, baseline perfusion and perfusion increases to hypercapnia (10% CO_2) were used as estimates of vascular function. No estimates of brain clearance were acquired with MRI.

Results: In wild type animals, faster tracer clearance was observed during visual stimulation versus during rest (18% lower tracer signal after 1 hour, p=0.02). Decreases in spontaneous vasomotion were observed early (~10 months old) in transgenic APP23 mice, and coincided with amyloid-β accumulation in the vessel wall. Baseline perfusion and perfusion responses to hypercapnia were significantly lower in transgenic mice only at 24 months old (109 versus 86 mL/100 g/min baseline perfusion, p=0.0056; 47% vs 10% perfusion increase to hypercapnia, p=0.016, in respectively wildtype versus transgenic animals).

Conclusion: Taken together, the results provide support for the hypothesis that a downward spiral between vascular dysfunction and reduced brain clearance is involved in CAA pathogenesis.

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Medin amyloid and its role in Alzheimer's disease and vascular aging: A potential therapeutic target

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Introduction: Medin amyloid, a fragment of the protein MFG-E8, is the most prevalent human amyloid found in the vasculature of individuals over 50 years of age. It aggregates in blood vessels of aging wild-type mice, leading to cerebrovascular dysfunction. In humans, medin aggregates are observed in the cerebral arterioles of patients with vascular dementia or Alzheimer's disease (AD). Given its prevalence and vascular localization, understanding medin's role in neurovascular dysfunction could provide valuable insights into AD pathogenesis. This study investigates the impact of medin amyloid on vascular amyloid-β (Aβ) deposition and explores its potential use as a biomarker and novel therapeutic target.

Methods and Material: We analyzed medin and Aβ colocalization in brain tissue from APP-transgenic mice and AD patients. Immuno-electron microscopy, co-aggregation assays, and structural modeling were used to examine medin-Aβ interactions. Medin-specific antibodies were developed to study the association with cerebral amyloid angiopathy (CAA).

Results: Medin was found to promote vascular Aβ aggregation in APP transgenic animals, as medin-deficient mice had substantial reductions in CAA. In patients, medin was also associated with vascular Aβ deposits, with higher levels correlating with increased Aβ accumulation. Moreover, increased *MFGE8* expression was linked to cognitive impairment, independent of other AD hallmark features. *In vivo* and *in vitro* assays further confirmed that medin aggregates accelerate Aβ aggregation. Structural modeling indicated direct interactions between medin and Aβ.

Conclusion: Our data strongly support medin amyloid as a driver of cerebral amyloid angiopathy, highlighting medin as a potential biomarker and novel therapeutic target for vascular dysfunction and cerebral amyloid angiopathy. These results warrant further evaluation of the role of medin in vascular pathology in our newly generated mouse lines modeling human medin pathology.

All necessary ethical approvals for this study have been obtained.

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GLP-1 and the cerebrovasculature

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Background/Aims: Vascular dementia/stroke is a leading, yet poorly understood cause of disability. The extensive cerebrovasculature that it affects plays a critical role in meeting the brain's energy demands, however there are currently limited treatments available to treat this pathology. The targeting of the signalling pathway of the hormone GLP-1, has been demonstrated to reduce stroke-associated morbidity, however the mechanisms of the pathway involved are still not fully understood. In this study, we aimed to investigate how GLP-1 signalling affects the function of the cerebrovasculature.

Methods: Stroke was induced in rats using the middle cerebral artery occlusion (MCAO) model. Native GLP-1 release was induced using remote-ischemic conditioning (RIC), involving 4 cycles of ischaemia of the hind limb, followed by reperfusion. A GLP-1 receptor reporter mouse (GLP-1R-CRE/tdRFP) was used to histologically identify the location of GLP-1 receptors on the cerebrovasculature. Cerebral arterioles were imaged in ex vivo acute brain slices under conditions of oxygen-glucose deprivation, mimicking ischemic stroke. The GLP-1 receptor agonist Exendin 4 (Ex4) was used to stimulate GLP-1 receptor signalling. Cerebral blood flow under Ex4 was measured using an Oxylite probe in vivo.

Results: RIC significantly reduced the area of infarct in the MCAO model and improved the neurological score. The specificity of the involvement of the GLP-1 pathway in this neuroprotection was confirmed by an abolition of this effect with administration of the GLP-1 receptor antagonist, Exendin-9. The presence of the GLP-1 receptor was found to be primarily on cortical arterioles. In cortical arterioles pre-constricted by oxygen-glucose deprivation, ex vivo application of Ex4 led to vasodilation. This dilatory effect was found to be mediated through the cAMP signalling pathway. In naïve rats, intravenous administration of Ex4 was found to increase cerebral blood flow.

Conclusion: The results of this study demonstrate that GLP-1 receptor signalling protects against ischemic stroke through a dilatory effect on cortical arterioles, which functionally leads to increased cerebral blood flow. The targeting of this pathway could therefore be beneficial against stroke/vascular dementia, and future plans involve investigating this effect in a mouse model of vascular dementia.

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Imaging microcirculation in a controlled in-vivo model of sepsis

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Background and aims: Sepsis is a severe medical condition in which both the macro- and microcirculation play a crucial role. A well-established method for studying the symptoms in a controlled setting is experimental endotoxemia, a model based on the sterile activation of the immune system through intravenous injection of an endotoxin, lipopolysaccharide (LPS). This will affect the function of the vascular endothelium, providing the opportunity to study both the onset and recovery from the infection-like state.

Recent advances in microcirculation imaging techniques – multi-exposure laser speckle contrast imaging (MELSCI) and multi-spectral imaging (MSI) – enables studying the spatiotemporal dynamics of the microcirculation. The aim of this study was to investigate changes in microcirculatory perfusion and blood oxygen saturation, as well as regulatory function using wavelet-based flowmotion analysis, during LPS injection.

Method: 17 healthy individuals were enrolled to two separate measurement days with randomized injection of either LPS or placebo in a crossover fashion. Measurements of speed-resolved perfusion and blood oxygen saturation were performed using a multimodal imaging system combining MELSCI and MSI. Facial microcirculation was recorded for 20 minutes at four timepoints; baseline (2h pre-injection), and T1.5, T3.5, T5 (hours post-injection). Median perfusion and oxygen saturation, as well as flowmotion activity, were computed in a region of interest on the forehead. Changes in measured parameters from baseline were analyzed.

Results: Perfusion decreased at T1.5 (median -30%, $p<0.001$), returned to baseline at T3.5, and increased above baseline at T5 (+22%, $p<0.01$). Oxygen saturation decreased at T1.5 (-7%, $p<0.001$), then returned to baseline at T3.5 and T5. Largest changes in flowmotion were observed in cardiac activity at T1.5 (-47%, $p<0.01$), T3.5 (-32%, $p<0.001$), and T5 (-23%, $p<0.05$), in neurogenic activity at T1.5 (+138%, $p<0.01$), and in endothelial activity at T1.5 (+102%, $p<0.01$), T3.5 (+83%, $p<0.01$), and T5 (+70%, $p<0.01$). Endothelial flowmotion was also increased after placebo at T1.5 (+93%, $p<0.01$) and T3.5 (+77%, $p<0.01$).

Conclusions: Significant changes in microcirculatory function were observed following LPS injections, which mostly were not measured after placebo. The combination of the LPS model and novel imaging system is promising for studying the microcirculation in shock.

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Advancing skin microvascular assessment: A comparative review of wavelet analysis

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Background: Laser Doppler flowmetry (LDF) is widely regarded as the gold standard for non-invasive assessment of skin microvascular function. The complex laser Doppler (LD) microcirculatory signal reflects various biological and physiological processes. To extract as many signal properties as possible, spectral analysis is recommended. Spectral analysis can be performed using classical Fourier transform (FT) or wavelet analysis (WA). While FT offers frequency analysis, it lacks time localization, whereas WA provides refined time and frequency localization, making it a more powerful tool for analyzing signal dynamics.

Methods: This review examines the application of WA in analyzing LD microcirculatory signals. WA has revealed six characteristic frequency intervals ranging from 0.005 to 2 Hz. These intervals are associated with specific physiological influences that modulate the skin's microcirculatory response, including endothelial nitric oxide (NO)-independent, endothelial NO-dependent, neurogenic, myogenic, respiratory, and cardiac factors. We contrast the advantages of WA with FT and explore the challenges and limitations of current methods in analyzing LD signals.

Results: WA of LD signals has provided a more comprehensive understanding of skin microvascular function by identifying the six frequency intervals linked to different physiological influences. WA allows a more nuanced interpretation of microcirculatory responses in healthy and diseased individuals. However, despite its advantages, WA still faces unresolved limitations, such as a lack of standardization in the analysis process and challenges in consistent interpretation across studies.

Conclusion: WA presents a valuable advancement over FT for assessing skin microcirculation, offering better time-frequency resolution and more detailed insights into microvascular function. However, the method’s limitations must be addressed to improve its clinical applicability. Enhancing WA techniques is critical for accurately assessing diseases characterized by microvascular dysfunction, and further research is necessary to optimize this approach for clinical use. This review summarizes the advantages, limitations, and future directions for WA in evaluating LD microcirculatory signals.

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Exploring microvascular reactivity with photoplethysmography

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Background/Aims: Photoplethysmography (PPG) is a non-invasive optical technique that detects variations in skin blood volume with each cardiac cycle. Due to its portability and affordability, PPG has gained increasing attention in wearable health monitoring. However, the physiological sources contributing to PPG signals remain a topic of debate. This study aims to assess the sensitivity of PPG to microvascular reactivity by analyzing spectral components during different vascular challenge tests.

Introduction: PPG signals reflect the dynamic interaction between multiple physiological processes, including cardiac output, respiratory activity, autonomic regulation, and endothelial function. Understanding these contributions is crucial for improving the interpretation of PPG data in clinical and research settings. By applying wavelet transform (WT), we aimed to decompose the PPG signal and evaluate the specific spectral changes associated with distinct vascular stimuli.

Methods and Material: A low-cost PPG system was used to assess microvascular reactivity in 30 healthy adults (15 per group). Each participant underwent a vascular challenge test on a randomly assigned upper limb while the contralateral limb remained in a fixed position. Group 1 performed unilateral limb dependency for 3 minutes to evoke the venoarteriolar reflex (VAR), while Group 2 underwent suprasystolic upper limb occlusion to induce post-occlusive reactive hyperemia (PORH). PPG signals were recorded from the fingers of both hands. Wavelet transform (WT) was applied to extract spectral components related to cardiac, respiratory, myogenic, sympathetic, and endothelial activity.

Results: Both limb dependency and occlusion led to a significant reduction in perfusion in the tested and contralateral limbs, with the latter showing a less pronounced decrease, likely due to sympathetic activation. WT analysis revealed distinct spectral alterations between the two vascular challenge tests, suggesting different underlying regulatory mechanisms.

Conclusion: PPG demonstrates sufficient sensitivity to detect subtle perfusion changes during vascular challenge tests. Spectral analysis using wavelet transform provides mechanistic insights into these changes, reinforcing the potential of PPG for microvascular assessment in both clinical and research applications.

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Microvascular reactivity in chronic kidney disease in human

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Background/Aims: Chronic kidney disease (CKD) is characterized by initially asymptomatic, but over the time progressive loss of kidney function leading to kidney failure. Among various etiological factors, diabetes and hypertension are the most frequent. CKD patients are at the increased risk of cardiovascular complications and mortality. While many studies were focused on macrocirculation, hereby, the novel findings in regard of microvascular function in CKD will be presented.

Introduction: CKD is characterized by a persistent inflammation that affects vasculature too, and presents a risk factor for endothelial dysfunction (ED). However, oxidative stress affects vascular reactivity and is considered as one of the most important causes of ED too, underlying all cardiometabolic diseases, including CKD. ED is a link between CKD and cardiovascular event, such as atherosclerosis, calcification of blood vessels and increased arterial stiffness on one side, and heart failure, left ventricular hypertrophy and atrial fibrillation on the other side, leading to increased mortality.

Methods and Material: A review of the recent literature, together with preliminary results of own cross-section study on microvascular function in CKD and hypertensive patients (Ethical committee approval #2158-61-46-24-121; ClinicalTrials.gov Identifier: NCT06316284) will be presented.

Results: Adverse alterations of the vascular function are mostly pronounced distal to the level of small arterioles. However, majority of studies focus on macrovascular complications, while microvascular complications and oxidative stress remain under-investigated. CKD patients, compared to healthy controls, exhibited reduced microvascular density in skeletal muscles and in the heart and increased oxidative stress biomarkers, associated with renal, hepatic, and coagulation failure, and weakened vasodilation. Functionally, our study showed impaired microvascular blood flow in response to endothelium-dependent stimulus, accompanied by altered biomarkers of oxidative stress and altered activity of some of antioxidative enzymes in CKD patients compared to hypertensive patients and controls.

Conclusion: Although microvascular reactivity is impaired in CKD patients, it is not given enough attention. Understanding of the pathophysiological mechanisms in ED, including the maintenance of oxide-antioxidative balance, may open new diagnostic and therapeutic possibilities and requires further research in CKD.

Funding: Medical Faculty of Osijek institutional grants IP18-MEFOS-2024 and IP20-MEFOS-2025 (PI I. Drenjančević)

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Microvascular over-dilation - a novel tissue level mechanism causing hypoxia and chronic tissue damage with reference also to skeletal muscle regeneration

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Background/Aims: The benefit or harm of ischemic microvascular remodeling is not completely understood and may limit also the potential of treating ischemic diseases that remain the leading cause of death worldwide. The aim of this research was thus to dissected the biological relevance and the pathomechanistic role of hypoxic microvascular remodeling.

Methods and Material: In a series of clinical and preclinical studies, among others high-resolution contrast enhanced ultrasound and photoacoustic imaging as well as immunohistological analyses of tissue samples were employed both in animal models of acute (n=103 mice) and chronic hindlimb ischemia (n=44 rabbits) and patients with chronic limb-threatening ischemia (CLTI) (n=90 patients) to study: 1) the natural course of microvascular changes leading to efficient muscle regeneration or chronic ischemic damage in animals; and 2) the functionality of ischemic muscle microvascular blood flow in humans.

Results: In animal models, post-ischemic capillary enlargement ($P<0.05$) was identified to facilitate collateral maturation and the recovery of arterial driving pressure after which an intussusceptive increase in capillary density ($P<0.05$) was needed to recover muscle oxygenation and support muscle regeneration. Furthermore, decreased capillary transit time ($P<0.01$) and a gradual arterialization of capillaries along worsening of ischemic damage ($P<0.001$) were observed in CLTI and induced chronic muscle damage also in healthy animals.

Conclusion: These results depict a critical role for the microvasculature determining muscle damage and regeneration. Importantly, an over-dilation of the skeletal muscle microvasculature impairs oxygen diffusion and therefore presents a novel cause for tissue hypoxia.

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The cardiac microvasculature in aging, obesity and exercise

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Background/Aims: Cardiometabolic diseases (CMD) are major health problems worldwide. Endothelial dysfunction is among the first derangements during CMD development. Recent data including those from my laboratory, intriguingly suggest that endothelial dysfunction is not only a consequence of CMD, but instead, dysfunctional ECs can be primary instigators of subsequent organ dysfunction. Although exercise is the most efficacious way to prevent CMD, the molecular mechanisms of its effects are still, perhaps surprisingly, insufficiently understood. We aim to unravel the role of endothelial cells in mediating the detrimental effects of CMD and the health benefits of exercise.

Methods and Material: We use CMD and aging mouse models with exercise and in vitro exercise-on-a-dish cellular models. The translational potential is evaluated in obese CMD patient cohort, who are treated with exercise. We use scRNAseq proteomics and imaging to study the effects of obesity, aging and exercise on endothelial cells.

Results: Our results show that aging, obesity and exercise markedly remodel microvascular endothelial cell transcriptomes in the heart, while arterial and venous EC transcriptomes are much less affected. We have identified cellular senescence and endothelial-to-mesenchymal as pathological processes induced by aging and obesity, while exercise training repressed both pathways in endothelial cells. We have also identified cell-cell communication pathways in the heart that are changed in CDM.

Conclusion: Aging and obesity significantly remodel cardiac microvascular endothelial cells and induce similar pathological changes. Interestingly, exercise training as a physiological stimulus affects many of the same pathways, but to the opposite direction.

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Circulatory disorders in type 1 diabetes type, from big to small vessels and vice versa

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Disorders of microcirculation are responsible for early but also for advanced vascular changes at multiple levels including such important organs as myocardium in which case macro- and microvascular impairment could have fatal consequences. Therefore, knowledge of pathophysiological processes responsible for disorders at multiple arterial territories and their interconnection including microcirculation is of high importance. Therefore, studying and describing these processes at experimental/physiological level might further stimulate clinical research leading to more effective treatment of wide range of vascular disorders. One example of particular population which could potentially profit from this multivessel diagnostic and management approach including detection of microvascular disorders are persons suffering from type 1 diabetes. Moreover, already today it is possible to detect microvascular dysfunction on everyday basis in particular patients in well-equipped angiology departments and offices.

Supported by the project National Institute for Research of Metabolic and Cardiovascular Diseases (Programme EXCELES, ID Project No. LX22NPO5104) - Funded by the European Union – Next Generation EU.

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Diabetes related microangiopathy assessment in the angiologist everyday practice

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Disorders of microcirculation are responsible for early but also for advanced vascular changes which could affect prognosis and quality of life of affected persons/patients. Recently wide range of clinical methods are tested with potential to detect microcirculation changes in addition to assessment of macrovascular disease are available. These include methods based on modern plethysmography, laser examination and others. Therefore, introduction of these methods in offices of angiologists could really help in therapeutic and management decision in population of patients and persons at risk for cardiovascular events. In particular, detection of microcirculation might in clinical settings substantially help in vascular assessment of particular patients, mainly diabetic type 1 and 2 but also other patients not with obvious vascular impairment but with metabolic, renal, systemic and other diseases carrying risk of microvascular disease. In our presentation we will focus on practical approaches how to diagnose and interpret these findings based on everyday clinical/office practice.

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Longitudinal functional ultrasound (fUS) imaging reveals the impact of dietary choices on cerebrovascular aging

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Background/aims: Aging is strongly associated with changes in neurovascular function and vascular architecture, significantly affecting cerebral blood flow (CBF) and cognitive health. These age-related cerebrovascular alterations contribute to vascular cognitive impairment and dementia (VCID). Diet plays a crucial role modulating the progression of age. Time-restricted feeding (TRF) has emerged as a potential strategy to improve cerebrovascular health, while high-fat diets (HFD) exacerbate vascular dysfunction and cognitive decline. However, the deep cerebrovascular structures and CBF in aging murine models have not been well studied due to technical challenges. This study aimed to assess neurovascular coupling (NVC), CBF, vascular density, and brain transcriptional differences between young (4-6 months) and aged (24 months) C57Bl/6 mice, including aged groups on TRF or HFD for 6 months.

Methods: We performed functional ultrasound (fUS) imaging by implanting a polymethylpentene (TPX) window in place of the skull in 5 mice belonging to each group to allow the visualization of deep cerebrovasculature. Cortex tissue from the same mice was used to perform single cell transcriptomic to reveal potential critical genes and regulatory mechanisms involved in the vascular remodeling.

Results: Our results showed that aged mice, particularly those on an HFD, had significant reductions in vascular density, CBF velocity, and flow rate compared to young mice. These changes impaired NVC, disrupting blood flow regulation in response to neuronal activity. Conversely, mice on TRF exhibited improvements in vascular density and CBF velocity, suggesting that TRF helps preserve neurovascular function and mitigates age-related declines in cerebral perfusion and cognitive performance. Transcriptional analyses revealed increased inflammatory markers and mitochondrial dysfunction in HFD mice, while TRF reduced inflammation and improved mitochondrial health, further supporting TRF's role in promoting metabolic and neurovascular resilience.

Conclusions: This study underscores the critical role of diet in regulating cerebrovascular health during aging. Our findings demonstrate that age-related neurovascular dysfunction, characterized by impaired NVC and reduced CBF, is exacerbated by an HFD, leading to vascular deterioration and increased inflammation. Conversely, TRF emerges as a potent intervention that preserves vascular integrity, mitigates metabolic dysfunction, and supports cerebrovascular resilience. These results highlight diet as a key modifiable factor influencing brain aging.

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Targeting cellular senescence to prevent cerebral microhemorrhages in aging

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Background/Aims: Cerebral microhemorrhages (CMHs) are increasingly recognized as critical contributors to vascular cognitive impairment and dementia (VCID). Aging exacerbates CMH susceptibility, partly due to the accumulation of senescent cells in the microvasculature, which leads to endothelial dysfunction, chronic inflammation, and blood–brain barrier disruption. The senescence-associated secretory phenotype promotes matrix remodeling and microvascular fragility, heightening the risk of CMHs. We aimed to investigate whether eliminating senescent cells with the senolytic agent could mitigate hypertension-induced CMH formation in aged mice and to develop a high-throughput analysis pipeline for CMH quantification.

Methods: Aged (18-month-old) and young (6-month-old) C57BL/6 mice were subjected to hypertension via angiotensin II and L-NAME administration. A subset of aged mice was treated with ABT263 to eliminate senescent cells. CMH burden was assessed using histological staining. A high-throughput machine learning-driven imaging pipeline was developed to enhance CMH quantification, incorporating color space transformation and random forest algorithm for whole-brain 3D reconstructions. Neurological function and gait abnormalities were evaluated using neurological testing and CatWalk gait analysis.

Results: Aged mice exhibited a significantly higher incidence and earlier onset of CMHs compared to young controls. Histological analysis revealed an accumulation of senescent endothelial cells, astrocytes, and microglia, coupled with elevated matrix metalloproteinase activity and oxidative stress markers. Treatment with ABT263 significantly reduced CMH incidence, delayed onset, and mitigated neurological impairments. The novel CMH quantification method demonstrated superior accuracy and efficiency compared to the traditional methods. Gait analyses confirmed that ABT263-treated mice exhibited improved interlimb coordination and reduced gait asymmetry.

Conclusion: These findings provide evidence that cellular senescence plays a key role in CMH pathogenesis by promoting microvascular fragility. Senolytic treatments effectively reduce CMH burden and associated functional deficits, supporting the potential of senolytics as a therapeutic strategy to prevent or alleviate age-related CMHs. Additionally, the implementation of a novel imaging pipeline represents a significant advancement in CMH quantification, offering a scalable and unbiased method for future cerebrovascular research.

Ethical Statement: All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Oklahoma Health Sciences Center.

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Senolytic therapy with Dasatinib and Quercetin mitigates focal stroke outcomes in the aging rat brain

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Background/Aims: Carotid artery stenosis, alongside aging, represents a major risk factor for acute ischemic stroke. Identifying modifiable risk factors allows for preventive therapeutic strategies in high-risk individuals. While senolytic treatment with dasatinib (a tyrosine kinase inhibitor) and quercetin (a flavonoid antioxidant) has shown benefits in various pathological conditions, its efficacy in preventing acute ischemic stroke remains unclear. Spreading depolarizations (SDs) serve as electrophysiological markers of cerebral injury severity and progression. This study investigates whether dasatinib and quercetin (D&Q) administration following carotid artery stenosis in rats confers neuroprotection against subsequent ischemic stroke by modulating SD characteristics and cellular senescence.

Methods: Carotid artery stenosis was mimicked in young (6–7 weeks) and aged (18–24 months) Wistar rats under isoflurane anesthesia by unilateral ligation of the right common carotid artery. Over the following two weeks, rats received intraperitoneal injections of either D&Q (dasatinib: 5 mg/kg; quercetin: 50 mg/kg) or vehicle every other day. Thereafter, focal ischemia was induced via distal middle cerebral artery occlusion (dMCAO) ipsilateral to the ligation. Spontaneous SDs were recorded for one hour in the parietal cortex using a glass capillary microelectrode, while cerebral blood flow (CBF) changes were assessed via laser speckle contrast imaging. Immunohistochemical analyses examined markers of cellular senescence and neuronal injury.

Results: D&Q treatment did not alter systemic physiological parameters (blood pressure, baseline CBF). Ischemia-induced CBF reduction occurred across all groups, with aged rats exhibiting higher susceptibility to spontaneous SDs. However, D&Q-treated aged animals showed significantly fewer SDs compared to vehicle controls. Furthermore, the repolarization slope, SD duration, and area under the curve—indicators of tissue recovery—were reduced in the D&Q-treated group. Immunohistochemical analysis revealed a significant decrease in senescent arteriolar smooth muscle cells, astrocytes and endothelial cells in the cortex, striatum, and hippocampus of D&Q-treated aged rats.

Conclusion: Recurrent SDs within the ischemic penumbra exacerbate neuronal injury. D&Q treatment post-carotid stenosis, but before ischemic stroke onset, mitigated SD recurrence, suggesting a neuroprotective effect. These findings highlight the potential of D&Q as a personalized, preventive strategy for patients diagnosed with carotid artery stenosis to limit stroke-related consequences.

Funding: H2020-No.739593, NKFIH-K134377, NAP3.0, TKP2021-EGA-28, SZAOK-Research Fund.

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Development of a comprehensive vascular assessment for a prospective workplace base cohort (The Semmelweis Study): contribution of retinal and peripheral vascular dysfunction to age-related vascular cognitive impairment

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Background/Aims: With a growing elderly population in the European Union, age-related diseases associated with unhealthy aging pose increasing public health challenges. The Semmelweis Study, an occupational cohort study in Hungary, seeks to identify determinants of unhealthy aging, focusing on the complex relationship between lifestyle, environmental factors, and the development of chronic age-associated diseases, including age-related vascular cognitive impairment (VCI). The objective of this pilot study was to establish a robust assessment methodology to evaluate peripheral and cerebrovascular health, and to investigate whether changes in vascular function are associated with cognitive impairment.

Methods: The study enrolled 49 healthy volunteers aged 23 to 87 years, who underwent cognitive testing with an automated cognitive test battery (CANTAB), examining memory, attention and psychomotor speed. Peripheral vascular health was comprehensively evaluated using laser speckle contrast imaging (LSCI), flow-mediated dilation (FMD), and measurements of vascular stiffness. The retinal microvasculature, which closely mirrors the cerebral circulation in anatomy, physiology, and pathophysiology, provided a unique window for examination. Static and dynamic retinal vessel analysis involved the measurement of retinal arteriolar diameter which allows detection of structural and functional changes in the central nervous system, which are highly relevant to the pathogenesis of VCI. The association of the collected retinal and cognitive measures were investigated and subsequently, the vascular variables were integrated into vascular health indices (VHI) with Principal Component Analysis, and the relationship to the age and cognitive status of study participants was explored.

Results: The ratio of retinal arteriolar and venular vessel diameter (AVR) was associated with the number of errors made during memory testing ($r=-0.476$; $p<0.01$). Retinal venular dilation was associated with the accuracy of responses during attention-psychomotor speed testing ($r=-0.476$; $p<0.01$). The VHI generated from peripheral and retinal vascular measures correlated with index generated from cognitive measures ($r=-0.53$; $p<0.05$).

Conclusion: The comprehensive vascular evaluation used in current study was able to identify associations between age-associated vascular dysfunction and multi-domain cognitive impairment.

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Racial disparities and microvascular function in cardio-oncology

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Background: Major-adverse-cardiovascular (CV) events (MACE) represent the leading cause of non-cancer related mortality among breast cancer (BC) survivors. Increasing evidence suggests decreased endothelial function is predictive of CTx induced cardiac events however the role of microvascular endothelial (dys)function, a well-established predictor of MACE, has been mostly ignored and few mechanistic studies exist humans.

CTx induced MACE are more prevalent in Non-Hispanic-Black (NHB) relative to non-Hispanic-White (NHW) patients. Despite the overwhelming clinical evidence supporting these health disparities, the underlying causes are poorly understood. The overall goal of this study is to determine possible racial differences in microvascular response to CTx that may contribute to clinical outcomes.

Methods: Isolated arterioles from BC patients (pre and post) and healthy donors after *ex vivo* exposure to CTx were us for video-microscopy. Endothelial function, underlying mediators and smooth-muscle function were compared between NHB and NWH patients. Vessels were used pre or ~1 month after cessation of CTx or incubated *ex-vivo* 16–20 h with CTx doxorubicin-Dox (100 nM), (trastuzumab-TZM (10mg/ml), or paclitaxel-PTX (1nM). Endothelial dependent flow-mediated-dilation (FMD 5-100cmH_2O pressure-gradient), acetylcholine (ACh 1nM–10 µM), and endothelial independent dilator papaverine (Pap 100µM) were evaluated. NO and mitochondrial derived H_2O_2 levels were quantified via fluorescent microscopy.

Results: No differences in endothelial function or production of NO/H_2O_2 were observed in micro-vessels from otherwise healthy patients of NHW vs. NHB background. BC patients treated with the cardiotoxic CTx (Dox and/or TZM) *in vivo* developed profound microvascular endothelial dysfunction. *Ex-vivo* exposure to Dox decreased endothelial function equally in vessels from NHB vs. NHW. TZM augmented endothelial dysfunction in vessels from NHB compared to NHW. PTX did not impair dilator capacity in NHW but showed a significant reduction in vessels from NHB. Papaverine responses were unchanged in either group.

Conclusion: BC patients undergoing treatment with cardiotoxic CTx develop microvascular endothelial dysfunction that is recapitulated in healthy arterioles exposed to CTx. Responses in vessels from NHB patients were augmented after *ex-vivo* exposure and suggesting racial disparities may contribute increased MACE. Causative events have to date not been identified and are likely influenced by biological stress responses, genetic predisposition and environmental factors.

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Spatial mapping of breast cancer tumour microenvironment in Black British and White British women

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Background/Aims: Women of Afro-Caribbean descent confront more aggressive breast cancer subtypes at a younger age than their Caucasian counterparts. Yet, breast cancer research and treatment development have predominantly focused on Caucasian populations, neglecting potential biological drivers of these disparities. Our study addresses this gap by in-depth characterising the breast tumour microenvironment (TME) in an ethnically diverse cohort.

Methods: We analysed treatment-naïve breast cancer samples from 45 Black British and 45 White British women, matched by age, tumour subtype, and stage by employing spatial transcriptomics (NanoString GeoMx) and hyper-plex protein profiling (Leica Microsytems Cell DIVE).

Results: We captured whole-transcriptome data from cancer (PanCK+), immune (CD45+), and stromal (aSMA+) compartments from both tumour centre and tumour edge. The most striking differences emerged within the immune and stromal compartments, not in the cancer cells, underscoring metabolic, adhesion, and extracellular matrix rewiring in Black British tumours. Complementary spatial protein profiling further revealed changes in tissue architecture with distinct recurrent patterns of cellular organisation and cell-cell interactions, involving endothelial and B-cells.

Conclusions: Our findings suggest that the TME plays a pivotal role in driving ethnic disparities in breast cancer, highlighting the urgent need for ethnically tailored therapies and more inclusive clinical trials to advance precision cancer care. This breakthrough offers new avenues for improving overall outcomes in breast cancer.

Ethical Statement: Human breast tissues were collected from consenting patients undergoing surgery at Barts Health NHS Trust London between 2000and 2015. Samples were stored and analysed with de-identied labels to protect patient data in accordance with data under the terms of the Barts Cancer Institute Breast Tissue Bank REC no: 15/EE/0192.

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Activation of the mtDNA-TLR9 pathway induces hypertension and endothelial dysfunction in pregnant rats, modelling key features of preeclampsia

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Background/Aims: Preeclampsia is a pregnancy-specific hypertensive syndrome associated with excess maternal innate immune system activation and vascular inflammation. Assessing a direct impact of innate immune system activation on maternal vessels, we examined whether stimulation of Toll-like receptor-9 (TLR9), a pattern-recognition receptor activated by mitochondrial DNA (mtDNA), disrupts vascular control during pregnancy using two complementary rat models.

Methods and Material: Sprague-Dawley rats received either (i) intraperitoneal injections of a synthetic TLR9 agonist ODN2395 (1 mg/kg/d) or saline from gestational day 14–20, or (ii) an intravenous bolus of purified hepatic mtDNA (350 ng/kg) or saline 4 h before tissue harvest. Blood pressure was recorded by radiotelemetry. Endothelium-dependent and -independent reactivity of mesenteric, uterine, and thoracic aortic rings was assessed by wire myography; vascular superoxide and eNOS coupling by fluorescence imaging and western blot.

Results: Chronic treatment with ODN2395 prevented the development of pregnancy-induced hypotension in late pregnancy ($p<0.05$ vs. saline), increased vascular superoxide and enhanced mesenteric, but not uterine, artery contraction to the thromboxane analog U46619. Acute mtDNA challenge impaired aortic acetylcholine relaxation and inhibited the anticontractile effects of the vascular endothelium, effects prevented by the TLR9 antagonist ODN2088. Uterine arteries and vessels from non-pregnant rats were unaffected in both models.

Conclusion: TLR9 activation, whether pharmacologic or driven by endogenous mtDNA, elicits endothelial-dependent, vascular-bed-specific dysfunction that manifests as hypertension during pregnancy. The absence of similar effects in non-pregnant rats underscores a pregnancy-specific vulnerability. These findings position the mtDNA/TLR9 axis as a mechanistic link between innate immune activation and maternal vascular pathology in preeclampsia and identify TLR9 antagonism as a promising therapeutic target.

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Piece of Heart - Piecing together cardiovascular disease mechanisms from *ex vivo* human cardiac tissue using multi-omics

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Background/aims: Numerous cell types and states make up a functional human heart. In health, these cells work together to accomplish the normal organ and tissue function, whereas in pathological states, small molecular disturbances may escalate into changes in cell proportions, signaling, and tissue function, ultimately leading to manifestation of symptoms and diagnosable disease. My research focuses on characterizing these cellular and molecular changes with omics approaches, with the aim of more informed translation of the biological findings into clinical applications.

Materials and methods: By mapping the molecular function of the *ex vivo* cardiac tissue with different omics technologies across cardiovascular disorders, my lab pieces together cardiovascular disease mechanisms disease-specifically and across diseases, gaining new insights into the human cardiovascular biology. We develop methods, gather data, build interactive gene expression networks, predict causal factors, decipher signaling molecules, and interpret the data in tissue context with spatial technologies.

Results: With our experimental and computational pipelines, we have established a computational cleanup for noisy biological data, built data resources for the field to utilize, enabled mechanistic explorations of disease-relevant changes in the human cardiac microvasculature and across various cell types, and identified potential markers and causal factors for the diseases, revealing molecules of interest for further testing. After analyzing >100 human samples, several lines of evidence suggest a critical role for the vascular cells in the cardiovascular disease onset and progression across disorders. The changes manifest far away from the traditional hotspots for the diseases, indicating a need for more data from different parts of the heart to understand the molecular effects the diseases have on cardiac health more broadly, and to pinpoint therapeutic targets that could help alleviate the pathological states and re-establish health.

Conclusion: The results highlight the potential of the multi-omics approaches in dissecting disease mechanisms, emphasize the need for more human-based data, and the inclusion of data from both men and women in the mechanistic explorations.

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Exploring the brain vascular function: from the pial vasculature to the delicate capillaries

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Our goal is to understand how blood flow dynamics are regulated in the complex of the brain vascular network in health and disease. A human brain is powered by ~650km of blood vessels which are compartmentalized into arteries, capillaries, and veins. This immensely complex network can easily fail during disease such as stroke.

We focus on the regulation of brain fluid dynamics with a special emphasis on blood flow recovery after ischemic stroke. Recently, our group has shown that pial collaterals are key components regulating reperfusion after stroke. Moreover, we uncovered that microvascular failure after stroke is due to capillary stalls by neutrophil plugging. Our group have been building novel imaging tools to study how to resolve obstructions in large vessels as well as in the microvasculature. We strive to find novel approaches to improve macro and microvascular function in order to keep the brain's circulatory system functional.

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Neurovascular uncoupling is linked to microcirculatory dysfunction in regions outside the ischemic core following ischemic stroke

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Background/Aims: Maintaining normal brain function relies on the ability of the vasculature to dynamically distribute blood flow to regions with high metabolic demand. Impairment in this neurovascular coupling may contribute to poor recovery and neurological outcomes following stroke despite recanalization.

Methods: Animals (n=6) were implanted with a half “crystal skull” cranial window that gave optical access to a whole hemisphere. They were then acclimated to head restraint so they could be awake for all experiments. A photothrombotic model of ischemic stroke was used targeting a distal branch of the MCA. Optical coherence tomography based angiography and laser speckle contrast imaging were used to measure capillary flow and neurovascular flow responses respectively. Capillaries and pericytes were studied *ex-vivo* via perfusion-fixation followed tissue by labelling with lectin and platelet-derived growth factor receptor β.

Results: We demonstrate that arterial occlusion led to contraction of capillary pericytes and a reduced number of perfused capillaries at 3 hour (45% [95% CI, 33%, 58%]) and 24 hour (53% [95% CI, 39%, 66%]) 24 hour post occlusion timepoints despite arterial reperfusion. We also observed a significant increase in dynamic “stalling” of capillary flow in the remaining perfused capillaries (0.5% [95% CI, 0.2%, 0.7%] at baseline, 5.1% [95% CI, 3.2%, 6.5%] and 3.2% [95% CI, 1.1%, 5.3%] at 3- and 24-hour follow-up, respectively; $P < 0.001$, $n = 6$). This dysfunction was associated with an impaired neurovascular coupling response to a whisker stimulus at the follow up timepoints.

Conclusion: Our data suggests pericyte induced capillary dysfunction as a potential contributor to poor stroke recovery,and is a potential therapeutic target to improve neurological outcomes. Ongoing work is investigating the time scale and extent of capillary dysfunction following the stroke, as it has key implications on any potential therapeutic window.

Funding: This work was supported by The International Network Programme by the Danish Agency for Science and Higher Education (nr.: 8073-00007B), The Independent Research Fund Denmark–Medical Sciences (8020-00084B), Lundbeck Foundation (R344-2020-952), and Helga & Peter Korning's Foundation (#2021-35).

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Distal embolization during clot dissolution as contributor to the no-reflow phenomenon

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Background/Aims: In ischemic stroke treatment it is critical to recanalize the occluded vessel, either by thrombectomy or thrombolytic therapy, in order to recover blood flow and preserve cerebral function. Many clinical and preclinical studies have investigated the effects of thrombolytic drugs on blood clot dissolution. However, little is known about the dynamics of clot dissolution and reperfusion during thrombolysis. In this study, we monitored in real-time how a fibrin-rich clot is dissolved, using a thromboembolic model of stroke in BALB/c mice.

Methods and Material: In the thromboembolic mouse model of stroke, an in situ clot was formed by injecting thrombin into the M2 segment of the middle cerebral artery (MCA). Clot dissolution was initiated 30 min post-stroke using intravenous t-PA. Two-photon laser scanning microscopy and laser speckle contrast imaging were used to monitor clot dissolution, vascular patency and blood flow recovery. Blood components (plasma, fibrin, platelets, neutrophils) were stained using intravascular dyes (Texas Red Dextran, Rhodamine 6G, Hoechst 33342).

Results: Clot dissolution during thrombolysis was monitored in real-time. We showed that the clot gradually fragmentizes and dislocates through the MCA from proximal to distal arterial branches and penetrating arterioles. The more the distal embolization progressed, the smaller the volume of tissue restricted blood flow got, i.e. while the clot was dislocating from the mother artery to distal arterial branches, the volume of the engaged microvascular network decreased, reducing the volume of ischemic tissue. At the same time, the number of capillary stalls by neutrophils in distal capillary networks was higher compared to proximal capillary networks, reflecting the gradual tissue reperfusion during clot dislocation.

Conclusion: Our findings suggest that distal embolization during clot dissolution may be a contributor to the no-reflow phenomenon in stroke. New ways of clot fragmentation and accelerated clot washout are needed.

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Continued dysfunction of capillary pericytes promotes no-reflow after experimental stroke in vivo

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Background/Aims: Pericytes regulate cerebral blood flow and are reported to impair microvascular flow after cerebral ischemia by dying while constricted around capillaries. However, *in vivo* evidence supporting this conclusion has remained elusive. Here, we used repetitive *in vivo* 2-photon microscopy to investigate whether pericytes survive ischemic stroke, constrict during ischemia, and are causally implicated in the no-reflow phenomenon upon reperfusion.

Methods and Material: NG2dsRedxPDGFRbEGFP mice with fluorescent pericytes underwent transient middle cerebral artery occlusion or sham surgery and pericyte survival, vessel diameter, and cerebral blood flow were assessed after stroke using *in-vivo* 2-photon imaging, immunohistochemistry, laser-speckle contrast imaging, and transcriptomic analyses.

Results: We show 87% of pericytes constrict during cerebral ischemia, remain constricted post-reperfusion and 50% of pericytes show morphological signs of hyper-acute membrane damage. Moreover, we reveal ischemic pericytes are fundamentally implicated in capillary no-reflow by arresting blood flow within 24 hours post-stroke. In the ischemic cortex, we observe pericytes remain viable, responding to the RhoA Kinase inhibitor Fasudil by dilating capillaries and releasing associated capillary stalls, before upregulating unique transcriptomic profiles tuned toward replication. Finally, we demonstrate ischemia differentially constricts pericytes in a depth-dependent and sub-type specific manner and their delayed recovery after reperfusion predicts long-term vessel reconstriction in the days following stroke.

Conclusion: In summary, our data show functional impairment of surviving pericytes dominates over cerebral pericyte death to mediate no-reflow following transient cerebral ischemia. Cumulatively, these findings demonstrate that surviving cortical pericytes remain both viable and promising therapeutic targets to counteract no-reflow after ischaemic stroke.

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Collateral is brain: Low perfusion triggers spreading depolarization and futile reperfusion after acute ischemic stroke

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Background/aims: The capillary no-reflow phenomenon refers to the inadequate microvascular perfusion despite technically successful recanalization in the acute ischemic stroke (AIS) care. In fact, capillary no-reflow, otherwise referred to as futile reperfusion, is associated with poor neurological outcomes in more than half of the treated AIS patients. Here we explore the role of the most prominent secondary pathological event, spreading depolarization (SD) in the development of capillary no-reflow.

Methods: AIS was induced by the bilateral occlusion of the common carotid artery (2-vessel-occlusion, 2VO) under isoflurane anesthesia (1.5-2%) in adult (3-5 months old) male and female C57BL/6 mice (n=57). After a brief baseline (15 min) AIS was induced for 45 minutes followed by a subsequent 60 minutes of recording. Cerebral blood flow (CBF) changes were monitored by Laser Speckle Contrast Analysis (LSCI) and SD propagation was visualized by the recording of intrinsic optical imaging. After 24 hours, the neurological deficit of mice was evaluated by the Garcia Neuroscore (GN) scale. Then, the animals were transcardially perfused with carbon-based ink to assess the primary collateralization of the Circle of Willis (CoW). SDs were blocked with the NMDA receptor antagonist MK801 (0.3 mg/kg bw).

Results: SDs emerged after AIS onset in one or both hemispheres below a perfusion threshold (CBF drop to 21.1 ± 4.6 vs. 33.6 ± 4.4%, SD vs. no SD). The no-reflow phenomenon was invariably linked to previous SD, in contrast, reperfusion was adequate in hemispheres devoid of SD (44.4 ± 12.5 vs. 98.9 ± 7.4%, no-reflow vs. normal reperfusion). Absence of the P1 segment of the posterior cerebral artery in the CoW favored SD occurrence and capillary no-reflow. SD occurrence and no-reflow were associated with poor neurologic outcome, and neuronal necrosis 24 hours after AIS. The inhibition of SD significantly improved reperfusion.

Conclusion: Our data provides substantial evidence to support the hypothesis that SDs play a pivotal role in the process of capillary no-reflow despite successful recanalization. The pharmacological inhibition of SD evolution is proposed as a potential therapeutic strategy to attenuate futile reperfusion and enhance cerebral blood flow following recanalization in AIS.

Acknowledgements: This work was funded by the EU H2020-HCEMM (No. 739593), NKFIH (No. K134377), FK142218, TKP2021-EGA-28, Nemzeti Agykutatási Program 3.0, SZTE SZAOK-KKA No:2024/5S777 A202 and the EKÖP-24-2-SZTE-297 scientific grants.

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About microcirculation and hemorheology in leukemia

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Background/Aims: Lymphoblastic leukemia is one of the severe diseases of the hematopoietic system. Long-term treatment is necessary, which is not always effective. Particular attention should be paid to blood cells and their motor function, which is described by the state of microcirculation and hemorheology.

Methods and Material: We investigate patients with lymphoblastic leukemia (n=56, age from 39 to 44). We used standard list of clinical analysis. And as part of the work, an algorithm was derived that demonstrates the relationship of morphometric features with microcirculatory and hemorheological properties of blood. The purpose of the algorithm was to study the parameters of the blood system using clinical tests and in silico calculations against the background of the clinical picture of the number and size of formed elements, lymphocyte formula of patients with lymphoblastic leukemia.

Results: In the group of patients with leukoblastic leukemia, the number of neutrophils was reduced by 40% compared to the control. Other types did not change significantly, thrombocytopenia developed, hemoglobin concentration and hematocrit index decreased. The number of erythrocytes did not change compared to the control. Dynamics

of the size of erythrocytes is an indicator of the lability of the blood system. The proportion of young erythrocytoid forms is small. The studies revealed changes in the geometric characteristics of erythrocytes. The increase in the overall dimensions (diameter and height) of erythrocytes in leukemia compared to the control changed by 20%. This indicates a change in microcirculation in leukemia.

Conclusion: Violation of blood fluidity occurs due to changes in erythrocyte aggregation, deformability of the erythrocyte membrane, plasma viscosity with an unchanged number of erythrocytes, i.e. with a normal hematocrit. Thus, in addition to standard studies in leukemia, it is necessary to monitor blood microcirculation by physical or in silico methods.

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Hemorheologycal study in patients with different form of atrial fibrillation

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Background: Currently, there are five types of atrial fibrillation or atrial fibrillation: newly diagnosed — regardless of the duration of the arrhythmia episode; paroxysmal — attacks are repeated, but the sinus (normal) rhythm is restored on its own. This usually happens within the first 2 days. Episodes can recur once every few years; persistent — atrial fibrillation bothers for more than a week. It may be necessary to restore normal rhythm with an electric shock (cardioversion) or antiarrhythmic drugs; long-term persistent — lasts more than a year with attempts to restore normal rhythm; permanent — lasts a year or more. Restoring sinus rhythm is ineffective, the body gradually adapts to atrial fibrillation. The goal of treatment is pulse control. With the help of medications, a heart rate (HR) is formed that is comfortable for life.

Materials and Methods: To understand of pathological processes this underlying, we aimed to access relations between hemodynamic and rheological properties of blood in patients with different forms. The erythrocyte (RBC) aggregation index (EAI), erythrocyte (RBC) deformability index (EDI) by original Georgian methods. 70 patients with an average age of 65±10 years because of various forms of atrial fibrillation were included in the study after final approvement of the study protocol by local EC and obtaining informed consents from all patients. There was no statistically significant difference between comorbidities. The cases of heart valvular disease, decompensated type 1 diabetes mellitus, and thyroid gland pathologies were excluded. The patients were distributed among control and 3 patients groups based on the type of atrial fibrillation. We used Origin 8.1 for statistics.

Results: The mean EAI was statistically higher in patients with paroxysmal, persistent, and permanent atrial fibrillation compared to the validation (control) group ($p=0.02$, $p=0.02$, and $p=0.01$, respectively); however, there were no significant differences between development groups. The highest mean EAI was in the group of patients with a permanent form of AF (42.6±10.4), and the lowest mean EAI was in the group of paroxysmal atrial fibrillation (32.8± 8.3, $p=0.02$). The mean EDI were statistically higher in all development groups compared to control ($p=0.01$, $p=0.02$, and $p=0.01$ for paroxysmal, persistent, and permanent atrial fibrillation patients, respectively), with highest indices in the group of patients with permanent atrial fibrillation (2.27±0.02) and lowest in the group of patients with persistent atrial fibrillation (2.20±0.05).

Conclusion: There were no significant differences between the groups of patients with atrial fibrillation. Therefore, it is advisable to prescribe the treatment regimen to patients with any form of arrhythmia against the background of blood hemorheology control

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Resistive index for studying microcirculation

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Background: Resistive arteries play a particularly important role, which they perform both under normal and pathological conditions. Being a kind of "taps" located on the border of macro and microcirculation, they participate in the regulation of the most important physiological parameters of the circulatory system. The development of various pathological and compensatory processes in the regional circulatory system and microcirculation is associated with resistive arteries. In view of all this, it is the resistive arteries of the circulatory system that should be the subject of diagnostic studies, as well as therapeutic interventions for various types of pathologies. All of the above indicates how important successful diagnostics of the functional state of resistive arteries in humans is, which should become the basis for effective therapeutic interventions that eliminate the corresponding disorders in patients. We have developed and tested a new principle for assessing the functional state of resistive arteries based on post-ischemic (reactive) hyperemia.

Materials and Methods: To determine the functional state of resistive arteries, we introduced the concept of "resistance index", which is the ratio of the area limited by the blood flow curve under normal (background) conditions and the zero line, to the area limited by the curve of increased blood flow during hyperemia and the zero line, over a time interval t equal to the period of return of the value of post-ischemic blood flow to its initial value. We examined 120 patients with arterial hypertension and a control group (n=18). The resistance index and erythrocyte aggregation were measured in all patients.

Results: The resistance index in patients increased by 50% compared to the control. This confirms a significant increase in the tone of their vascular walls along with hemorheological changes in arterial hypertension.

Conclusion: Continued research in the same direction may allow us to better understand the pathogenesis of various types of pathologies in the circulatory system, as well as use this technique to study other diseases associated with dysfunction of the resistive arteries.

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Development of a hybrid fluid-structure interaction (FSI) algorithm for red blood cell microcirculatory deformation under shear stress, and hypothesized O_2-dependent ATP release

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Background: The microcirculation is critical for oxygen (O_2) delivery to tissues, as red blood cells (RBCs) navigate through capillary networks. While imaging techniques provide insights into O_2 saturation and content within capillaries, methods to quantify O_2 transfer from capillaries into surrounding tissues remain limited. Consequently, mathematical modeling has become an essential tool for investigating how O_2 is distributed in response to dynamic metabolic demands and for elucidating mechanisms regulating capillary blood flow. RBCs, omnipresent in the microcirculation, have been implicated in capillary-level signaling mechanisms that modulate upstream arteriole tone, thereby influencing capillary perfusion. Specifically, RBC deformation and oxyhemoglobin desaturation have been shown to stimulate the release of adenosine triphosphate (ATP), a potent vasodilator. It has been hypothesized that ATP release in response to RBC mechanical deformation serves as a feedback mechanism to optimize microvascular oxygen delivery, yet a comprehensive model is required to systematically investigate this phenomenon. To address this, a condensed matter model of RBC deformation was previously developed, capable of predicting RBC shape dynamics in the absence of blood flow at the micron scale. This model was designed to quantify RBC-generated forces on the surrounding microvascular environment, considering the cell's unique elastic properties.

Methods: To examine RBC elasticity in flow, a hybrid fluid-structure interaction (FSI) algorithm was formulated using a Discrete Exterior Calculus (DEC) framework. This novel approach models RBC deformation under physiologically relevant shear stress rates [1/ms], solving RBC-fluid interactions using DEC for both fluid and structure components, enabling simulations in complex vascular geometries, and making it the first of its kind.

Results: The algorithm tracks RBC shape changes in response to shear forces (%), osmolarity shifts, and membrane tension. Simulations reveal RBC deformation and shear stress variations across diverse microvascular geometries, providing insights into microcirculatory function.

Conclusion: Future work includes validation against in vivo data and an investigation of the relationship between RBC shear, and induced ATP release. This model advances understanding of RBC mechano-transduction and its role in microcirculatory autoregulation, with implications for pathological conditions affecting oxygen transport and hemodynamics.

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QiShenYiQi pills inhibited retinal microvascular hyperpermeability in db/db mice

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Objective: Diabetic retinopathy (DR) is a leading cause of blindness in adults worldwide. The present study aims to evaluate whether QiShenYiQi pills (QSYQ), a traditional Chinese medicine, can suppress diabetic retinal microvascular leakage in db/db diabetic mice.

Methods: C57BL/6 and db/db mice at the age of 6 (pre-treatment groups) or 8 (post-treatment groups) weeks were orally administered with low (0.25 g/kg), medium (0.50 g/kg), and high (1.00 g/kg) dose of QSYQ until 14 weeks old, respectively. FITC-dextran leakage and fluorescein sodium leakage, retinal morphological changes, retinal microvascular obstruction were evaluated; proteomics were used to find potential targets of QSYQ; the expression of Claudin-5, Occludin, ZO-1, JAM-1, VE-cadherin, ATP5α, ATP5β, ATP5D, VEGFR2, Ndufs3, Ndufb8, Acad9, Septin6, Tubb6, Dnm1, MMP2/9 and reactive oxygen species (ROS) were detected.

Results: Both pre- and post-administration of QSYQ could alleviate retinal microvascular leakage in db/db mice. Post-administration with a medium dose of QSYQ could reduce retinal microvascular blockage, suppress the downregulation of endothelial cell junction proteins Occludin and Claudin-5, and suppress the elevated expression of VEGFR2, MMP2/9 and the ROS levels. Proteomics analysis and further validation proved that, QSYQ inhibited the downregulation of cytoskeleton-related proteins Septin6 and Dnm1, and mitochondrial respiratory chain complex I related proteins Ndufs3 and Acad9 in retinal tissue of db/db mice.

Conclusion: The present study demonstrated that administration of QSYQ could attenuate retinal microvascular hyperpermeability in db/db mice, providing evidence for the clinical use of QSYQ in preventing retinal microvascular leakage in diabetic patients.

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Ginseng and Panax Notoginseng formula promotes post-stroke neurogenesis and neurological functional recovery through improving mitochondrial function and modulating oxidative redox signaling pathway

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Background: Ginseng and Panax notoginseng Formula (GPF) is a Chinese medicinal formula for health promotion but its application for ischemic stroke remains unknown. In this study, we tested the hypothesis that GPF could promote neurogenesis and neurological functional recovery through promoting mitochondrial function and modulating oxidative redox signaling pathway.

Methods: We firstly performed chemical identification studies by using QIT-TOF-MS technology. We the performed *in vivo* and *in vitro* experiments to verify the hypothesis. We investigated the effects of GPF (225 mg/kg, 450 mg/kg, 900 mg/kg per day) on inducing neurogenesis and improving the recovery of the neurological functions in transient middle cerebral artery occlusion (MCAO) ischemic mice in vivo. The C57BL/6N mice were anesthetized and subjected to 2 hours of MCAO ischemia plus 14 and 21 days of reperfusion. Morris water maze test, rotarod test, open field test and NIH neurological deficit scores were performed to evaluate the neurological functions. Neurogenesis was detected by using different biomarkers. We further investigated the neurogenesis-promoting effects of GPF on cultured neural stem cells under oxygen-glucose deprivation (OGD) *in vitro*. Mitochondrial dynamics and functions and oxidative redox signaling pathways were tested in vivo and in vitro.

Results: We tentatively characterized 9 compounds from the GPF extractions. GPF effectively reduced infarct sizes, improved neurological deficit scores and locomotor ability and enhanced the learning and memory capability in the transient MCAO ischemic mice. GPF treatment promoted neural stem cell differentiations in the subventricular zone (SVZ) and subgranular zone (SGZ) of the MCAO mice. BYC also up-regulated the expression of Aconitase 2 (ACO2), Succinate dehydrogenase complex, subunit A (SDHA), phosphorylation of AMP-activated protein kinase (p-AMPK), protein kinase B (p-Akt) and glycogen synthase kinase 3β (p-GSK3β), Sirtuini in the ischemic brains. GPF significantly improved the mitochondrial functions and increased the production of ATP, and promoted neuronal differentiations in the neural stem cells under OGD condition. GPF revealed to modulate multiple oxidative redox signaling pathways.

Conclusion: BYC could promote neurogenesis and neurological functional recovery in post ischemic brains by regulating the mitochondrial functions and oxidative redox signaling pathway.

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Traditional Chinese medicine Yiqifumai injection ameliorates LPS-induced microvascular hyperpermeability in multiple organs through energy metabolism

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Objective: Yiqifumai injection (YQFM) is a compound Chinese medicine used to treat microcirculatory disturbance-related diseases clinically. This study aimed to investigate whether YQFM and its main ingredients attenuated microvascular hyperpermeability in multiple organs and underlying mechanisms.

Methods: Rats were challenged by lipopolysaccharide (LPS) infusion (5 mg/kg/hr) for 90 minutes. YQFM (160 mg/kg/hr), ginsenoside Rb1 (Rb1, 5 mg/kg/hr), schisandrin (Sch, 2.5 mg/kg/hr), and Rb1 (5 mg/kg/hr)+Sch (2.5 mg/kg/hr) were infused 30 minutes before (pretreatment) or after (post-treatment) LPS administration. In in vitro experiment, human umbilical vein endothelial cells (HUVECs) were incubated with LPS (100 ng/ml) for 90 minutes. YQFM (1 mg/ml), Rb1 (100 μM), Sch (100 μM), or Rb1+Sch (200 μM) was added 30 minutes after LPS stimulation.

Results: Both pretreatment and post-treatment with YQFM attenuated cerebral venular albumin leakage during LPS infusion and cerebrovascular hyperpermeability at 72 hours after LPS infusion. Meanwhile, YQFM and Rb1+Sch inhibited LPS-induced mesenteric venule hyperpermeability, suppressed microvillar erosion and submucosal edema. Mechanistically, YQFM and Rb1+Sch restrained the decreased junction protein expression, ATP content, and mitochondria complex activities. Moreover, YQFM and its main ingredients inhibited toll-like receptor-4 (TLR-4) and ROCK1 expression, as well as Src and RhoA phosphorylation. Its main ingredients Rb1 and Sch worked synergistically, with Rb1 being more effective for ROCK/RhoA activation, while Sch attenuating TLR-4 expression and Src phosphorylation.

Conclusion: YQFM exerts a protective and ameliorated effect on microvascular hyperpermeability in multiple organs, possibly due to the interaction of its main ingredients through energy metabolism.

Key words: energy metabolism; hyperpermeability; lipopolysaccharide

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Microvascular recanalization by embolus extravasation in a rodent model of silent brain infarcts

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Background: Micro-embolus lodging in the cerebral micro-circulation prior and during an acute ischemic stroke may contribute to the lack of microvascular reperfusion after treatment and is a potential factor in poor patient outcome. Micro-emboli can be cleared by endogenous fibrinolysis or by the lesser-known process of angiophagy, where occluding particles are extravasated via endothelial transmigration. However, the mechanisms of embolus extravasation remain poorly studied. A better understanding of embolus clearance may reveal new approaches to improve reperfusion after stroke. We investigated the mechanisms of angiophagy in a rodent model of silent brain infarcts.

Methods: We injected fluorescent microparticles (10 µm) whose lodging and extravasation were followed in vivo using two-photon microscopy for up to 28 days. In addition, molecular and cellular mechanisms of angiophagy were studied in an in vitro blood-brain barrier (BBB) model using human cerebral microvascular endothelial cells (hCMEC).

Results: Two-photon imaging observations showed reduced blood flow, transient BBB leakage, local and regional vascular diameter changes, and angiophagy. Vessels with a diameter of approximately 5 to 7 µm substantially expand distally from the microparticles, allowing 10 µm particles to slowly pass through the capillary network and extravasate within a few days. In vitro findings revealed actin-fiber remodeling, leading to endothelial engulfment and pore formation with actin rings tightly surrounding the microparticle. Engulfment of the particles led to upregulation of VE-cadherin (cells with vs without microparticles, $p<0.001$) and CD31 expression (cells with vs without microparticles, $p<0.001$) compared to the control group without microparticles.

Conclusion: We found preliminary evidence for large plasticity of the cerebral microvasculature, allowing 1) local widening preceding the movement of microparticles along the capillary network and 2) removal of large particles through the vascular endothelial wall via angiophagy, restoring perfusion.

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Stroke across scales – Impact on blood perfusion and oxygen supply in the brain

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Background/Aims: Microvascular disturbances are common during neurological disorders. On the smallest scale, we assess the relevance of single capillary occlusions (*microstroke*) for perfusion and oxygen availability. On a larger scale, we quantify the role of pial collaterals in maintaining perfusion during ischemic stroke.

Methods: To address these questions, we employ in silico flow and transport modeling in realistic vascular geometries. Our blood flow model accounts for the motion and impact of red blood cells. For the oxygen model, we consider intravascular oxygen transport bound to hemoglobin and dissolved in plasma. In brain tissue, oxygen diffuses and is consumed.

Results: Our microstroke simulations show that the drop in perfusion is strongly affected by the local vascular topology at the site of occlusion. Interestingly, the most robust local topology is also most frequent across the capillary bed. While oxygen availability is not significantly affected by the occlusion of a single capillary, an accumulation of occlusions causes a notable drop in oxygen partial pressure up to 75 µm from the site of occlusion.

To quantify the isolated impact of collaterals between the middle cerebral and anterior cerebral arteries, we use semi-realistic microvascular networks that only differ in the number of collaterals. The integral flow reaching the capillary bed for stroke conditions is 6 and 4 times larger for the configurations with 100% and 50% collaterals in comparison to no collaterals. This provides evidence that a higher number of collaterals causes an increase in residual perfusion in the infracted MCA-territory.

Conclusion: We show that even the smallest level of microvascular disturbances can impair the brain's energy supply locally. Moreover, both in the context of micro- and ischemic strokes, we highlight the relevance of vascular variability for the level of flow reduction. Both observations are directly relevant to understanding disease origins and therapeutic success and go beyond what is accessible to biomedical imaging alone.

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The histone deacetylase inhibitor suberoylanilide hydroxamic acid promotes blood-brain barrier protection during reperfusion in a cell culture model of ischemic stroke

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Background/Aims: Ischemic stroke is a leading cause of death worldwide with limited available treatment options. During ischemic stroke, reduced blood flow causes severe neuronal damage and BBB disruption. Thrombolytic therapy with recombinant tissue plasminogen activator (rtPA) which acts by breaking down clots to restore blood flow are the only FDA approved treatment for ischaemic stroke. However, rtPA is effective within a limited time (4.5 h) of symptom onset with a risk of intracranial hemorrhage. Therefore, additional effective therapeutic approaches for treatment of ischaemic stroke are urgently needed. The aim of this study was to investigate the effect of suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor, on restoring the function of the BBB to prevent post-stroke consequences.

Methods: We tested the effect of SAHA on human co-cultured BBB model under normoxia, oxygen-glucose deprivation (OGD), and during reperfusion after OGD (OGD/R).

Results: Our results show that after OGD, SAHA is able to prevent the BBB functions by increasing the resistance, and reducing the permeability of marker molecules across the BBB. SAHA increased the viability of pericyte cells against injury, which were much more damaged than brain endothelial cells. RNA-seq analyses showed that SAHA decreased the expression of genes involved in inflammatory processes and cell proliferation and increased the expression of the genes of glycocalyx enzymes.

Conclusion: Based on our results, SAHA may be a potential therapeutic tool for the treatment of ischaemic stroke, but further research is needed to gain a deeper understanding of its mechanism of action and to develop its potential clinical applicability.

A.S. was supported by Gedeon Richter Talentum Foundation. S.V. was supported by the Young Researcher Excellence Program (FK 143233) by National Research, Development and Innovation Office of Hungary. G.P. was supported by the National Academy of Scientist Education (FEIF/646-4/2021-ITM_SZERZ).

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The role of polycystin-1 in endothelial dysfunction and cardiovascular disease

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Introduction: Haemodynamic wall shear stress (WSS) exerted on endothelial cells (ECs) by flowing blood determines the spatial distribution of atherosclerotic lesions. ECs are able to sense and respond to WSS thanks to mechanoreceptors on their surface, which convert mechanical forces into biochemical signals. Among EC mechanoreceptors are polycystins (PKD1 and PKD2), causative genes for autosomal dominant polycystic kidney disease, associated with cardiovascular abnormalities by still unknown mechanisms.

Methods: Zebrafish embryos were used for functional screening of EC mechanoreceptors. Atherosclerotic plaque development was assessed in mice with inducible, endothelial specific deletion of genes of interest. PKD1 downstream targets were determined using single cell RNA sequencing of ECs isolated from mouse aortas. Candidate gene function was validated in human arterial ECs using small interfering RNA.

Results: A functional screening of mechanoreceptors in zebrafish revealed PKD1 and PKD2 as anti-apoptotic factors in ECs in response to haemodynamic forces. Knockdown of PKD1 or PKD2 in human ECs was pro-apoptotic. In mice, inducible, EC-specific loss of PKD1, but not PKD2, resulted in enhanced atherosclerosis. To dissect the underlying mechanisms, we performed single cell RNA sequencing of ECs isolated from PKD1-deficient and control mice and identified a number of downstream signalling targets, which we validated in vivo and in human ECs.

Conclusion: By integrating in vivo and in vitro models for studying EC responses to flow with –omics approaches, we identified PKD1 as novel shear-sensitive regulator of EC survival and a protective factor against atherosclerosis development. This has important implications for potential therapeutic approaches for atherosclerosis.

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Ultrasound and microbubble-induced blood-brain barrier opening: structural integrity and permeability assessment

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Background/Aims: The blood-brain barrier (BBB) tightly regulates molecular transport in and out of the brain but becomes compromised with age and neurodegenerative diseases. However, studying the systematic effects of BBB dysfunction is challenging due to the lack of reliable and inducible models. This study explored preclinical ultrasound (US) combined with microbubbles as a model to induce BBB opening in the whole mouse brain. This BBB opening was characterized via transmission electron microscopy (TEM), IgG staining, and fluorescent tracers to study endothelial damage, leakage areas, and BBB permeability respectively.

Methods and Material: Anesthetized mice received an IV infusion of SonoMAC microbubbles while power Doppler US was applied transcranially to induce BBB opening. To assess endothelial integrity, Evans Blue dye was injected post-BBB opening, followed by TEM analysis. Leakage areas were examined using an IV injection of lectin for vessel localization, followed by IgG immunostaining for leakage quantification using confocal microscopy. BBB permeability will be further evaluated using fluorescent dextrans of various sizes at different time points post-opening, followed by confocal microscopy imaging.

Results: TEM analysis confirmed BBB opening without overt endothelial damage. Ongoing analyses aim to quantify tight junction density and endothelial vesicle formation. Preliminary IgG staining revealed widespread tracer leakage, particularly around larger vessels, suggesting heterogeneous permeability changes. Further tracer studies are in progress to determine molecular size-dependent BBB permeability and temporal leakage dynamics.

Conclusion: US-induced BBB opening with microbubbles presents a promising model for studying BBB dysfunction in a controlled and non-destructive manner. Further characterization will clarify its potential for investigating BBB-related neurobiological changes, including those associated with neurodegenerative diseases.

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The role of protein acetylation in organ dysfunction during sepsis and the effect of SIRTs

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Background/Aims: Over the past decade, part of our research has focused on the role of activity alterations in deacetylase SIRTs during the development sepsis-induced organ failure.

Introduction: Post translational modifications (PTM), one of the important ways for cellular regulation of protein function, can alter the protein structure, stability, activity, localization, and interactions with other molecules by phosphorylation, acetylation, methylation, ubiquitination, glycosylation, lipidation, redox modifications, etc. PTM participates in almost all biological processes such as signal transduction, cell cycle regulation, and stress response.

Methods and Material: The cecal ligation and puncture (CLP)-induced septic mouse model was applied for in vivo studies and LPS-treated cells was use for in vitro studies. The expression and acetylation of proteins were detected, while the activity of deacetylase SIRTs was also measured. siRNA, overexpressing or mutant adenoviruses were applied to intervene the expression and activity of specific proteins. The morphology of mitochondria, specific cellular responses and organ function were also observed.

Results: The studies have revealed that the down-regulation of Sirt1, Sirt3, and Sirt5 in different cellular compartment induced the over acetylation of various target proteins, such as p53, HMGB1, PDHA1 and CypD, as well as Opa1, leading to the attenuation of autophagy, enhancement of inflammation, lower production of ATP and mitochondrial dynamic imbalance. These protein acetylations played critical role in sepsis-induced cellular dysfunction and subsequent organ failure. Our serious studies also proved that Melatonin, as well as Polydatin, a monomer of traditional Chinese medicine and isomer of Resveratrol, could attenuate the acetylation level of various target proteins by activating SIRTs, resulting in organ protection.

Conclusion: The down-regulation of Sirts and subsequent elevated acetylation of target proteins participate in the development of sepsis-induced organ dysfunction. Melatonin and polydatin preserve the organ function by activating SIRTs.

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Mechanisms of microvascular hyperpermeability and therapeutic targeting

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Microvascular hyperpermeability underlies a diverse spectrum of disease conditions, such as edema, myocardial ischemia-reperfusion (MI/R) injury, and psoriasis. Despite extensive research and therapeutic development for microvascular hyperpermeability, its molecular mechanisms remain unclear. We uncover that prostaglandin (PG) E_2, through acting its Gs protein-coupled receptor EP4, reduces microvascular hyperpermeability in MI/R injury and constrains MI/R injury in mice. Supplementing arachidonic acid (AA), the substrate for PG biosynthesis, reduced MI/R injury, and its combination with aspirin provided further cardioprotection. This is attributable to the selective augmentation of PGI_2 and PGE_2 that is amplified by thromboxane A_2 suppression by aspirin, underscoring improved microcirculation and ameliorated inflammation. In addition, we identified a downstream target—phosphodiesterase subtype 4B (PDE4B), which specifically hydrolyzes intracellular cyclic adenosine monophosphate, as a novel regulator of coronary microcirculation. PDE4B promoted coronary microcirculatory obstruction and vascular permeability in MI/R. Genetic deletion or pharmacological inhibition of PDE4B improved cardiac microvascular perfusion and suppressed neutrophil inflammation, preventing heart failure after MI/R injury. Factor XII (FXII) is the zymogen of the plasma protease FXIIa that activates the intrinsic coagulation pathway and the kallikrein kinin-system. We developed a single-domain antibody (Nb-Fc) that recognizes FXII in a conformation-dependent manner and interferes with FXIIa formation. In a mouse model of extracorporeal membrane oxygenation, FXII inhibition or knockout decreased thrombus deposition on oxygenator membranes and ameliorated organ injury, consistent with reduced systemic microvascular thromboinflammation, including microvascular neutrophil adherence, platelet aggregation and their interaction. Activation of FXII also promotes psoriasis via bradykinin, which critically mediates psoriatic microvascular inflammation. Nb-Fc treatment or FXII knockout markedly ameliorated psoriasis in mice. Thus, prostaglandins, PDE4B, and FXII are key regulators of microvascular hyperpermeability and bear promise for therapeutic targeting of microcirculation disorders.

Key words: microcirculation, hyperpermeability, signaling

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Diet-induced improvements of microvascular endothelial function: role of walnuts in maintaining membrane integrity

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Background: Cardiovascular diseases (CVDs) are the leading causes of mortality worldwide. One of the first line of defense against CVD is a healthy endothelium. Before considering pharmacological treatments, medical guidelines recommend lifestyle changes including dietary modifications. Indeed, a healthy diet has an important role in delaying the development of many modifiable risk factors of CVD, including abdominal obesity, high blood pressure, high plasma levels of cholesterol, and hyperglycemia. It has been shown that polyunsaturated fatty acids, phenolic compounds, and vitamin E are beneficial against CVD, and that walnuts contain many of these essential ingredients. Thus, we hypothesized that walnuts consumption improves that state of CV system.

Methods: The available literature was searched in PUBMED to investigate 1) the components of walnuts 2) the protective role of walnut consumption on CVD at large and 3) the potential cellular and molecular mechanisms underlying their beneficial effects on vascular endothelial function.

Results: The consumption of various nuts, especially walnuts, seem to benefit both primary and secondary prevention of CVD due to their bioactive components (such as polyunsaturated fatty acids, phenolic compounds, and vitamin E). These compounds have beneficial effects on vascular endothelial function and promote healthy membrane function. Since membranes are involved in nearly all processes associated with life-related function, the main underlying mechanism of walnut-improved cardiovascular function is likely through improving membrane composition and function. Walnuts provide essential substrates for membranes, including those of cells, mitochondria, Golgi, nucleus, and so on. In addition to endothelial cell function, all other cells and membranes are likely to benefit from walnut consumption.

Conclusions: The literature findings suggest that incorporating walnuts into the human diet may help mitigate the risk of developing cardiovascular diseases and compensate for the sedentary lifestyle particularly in older age. Walnuts consumption may be beneficial during higher physical and mental demand, such as exercise.

Support: National Research, Development and Innovation Office Hungarian University of Physical Education and Sport Sciences Thematic Excellence Program TKP2021-EGA-37, HAS/MTA Post-Covid 2021-34, OTKA K132596_19.

Keywords: cardiovascular disease, PUFA, phenolic compounds, mitochondria, mental function, exercise, sedentary lifestyles.

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The role and mechanism of traditional Chinese medicine in improving microvascular hyperpermeability

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Plasma albumin and fluid leakage due to microvascular barrier damage is the pathological basis of many edemas. The treatment based on the theory of crystal osmolality and colloidal osmotic pressure is not clinically satisfactory.

Plasma albumin and fluid leaked through capillaries and post-capillary veins are mainly mediated by the paracellular pathway mediated by the endothelial gap junctions and the transcellular pathway mediated by the caveola of vascular endothelial cells. Oxygen and nutrients are cycled through the mitochondria of vascular endothelial cells to produce NADH and FADH2, which are transmitted through the electron transport chain of mitochondria to produce ATP. ATP promotes the assembly of G-actin into F-actin, which supports tight junction proteins and adhesion junctions between vascular endothelial cells, and supports gap junctions between vascular endothelial cells. On the other hand, the high expression and phosphorylation of Caveolin-1 are regulated by Src.

The author confirmed that QiShen YiQi Dripping Pill, a compound traditional Chinese medicine composed of Astragalus membranaceus, Salvia miltiorrhizae, Panax notoginseng and Jianxiang, could improve the leakage of cardiac microvessels and cerebral microvessels caused by ischemia and reperfusion, and this effect was related to improving the mitochondrial respiratory chain of vascular endothelial cells, increasing ATP content, inhibiting the depolymerization of F-actin, maintaining vascular endothelial cell gap junctions, and inhibiting the phosphorylation of Src and plasma membrane Caveolin-1.

This study revealed the mechanism by which QiShen YiQi Dripping Pill improves microvascular hyperpermeability and edema by maintaining the microvascular barrier.

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Detection and quantification of vessel morphology on retinal fundus images – challenges of retinal vasculometry at scale

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Retinal fundus imaging provides a non-invasive way to observe the blood circulation of the body. The structure and appearance of retinal blood vessels have been linked to the early detection of systemic conditions, including cardiovascular disease. As a result, the eye serves as a valuable indicator of overall health, offering biomarkers that relate to both ocular disorders, such as glaucoma and diabetic retinopathy, and systemic diseases like diabetes and cardiovascular disease.

Systems for obtaining quantitative measurements of vessel morphology have been proposed in the literature, leveraging fully automated deep learning pipelines. These analytical approaches have played a significant role in epidemiological studies. Recent research has demonstrated that risk scores derived from the QUARTZ retinal vasculometry system can predict circulatory mortality, heart attack, and stroke with a performance comparable to established risk prediction models currently in use within health systems.

Retinal vasculometry approaches rely on core modules that utilise deep learning architectures to segment and quantify retinal features such as the optic disc and the vessels (categorised either as arterioles or venules). Modules for vessel analysis provide quantification of vessel features such as width and tortuosity. Ground-truth images, representative of a range of image characteristics, are used for training and subsequent validation of system performance to achieve reliable and robust vascular morphometric measurements.

The challenges encountered by retinal vasculometry systems relate to their specific use and area of application. Analysis of a diverse range of over 1.4 million images has recently been undertaken using QUARTZ. Challenges relate to generating reliable outputs across datasets containing images that represent diverse demographics, a wide spectrum of disease, a range of camera types and configurations and different image capture protocols. Various methodologies can be considered to assess image quality, characterized either by clinical demands or epidemiological requirements. A key aim in this context is to maximize the useful data extracted from large cohort studies, which may include a significant number of poor-quality images. Whilst end-to-end deep learning disease prediction models continue to gain prominence, retinal vasculometry offers interpretable results, identifying specific vascular features and changes which predict disease status.

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Microcirculatory responses to spreading depolarization in patients during neurocritical care

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Spreading depolarization (SD) is characterized by abrupt, near-complete breakdown of the transmembrane ion gradients, neuronal intracellular edema, excitotoxicity, activity loss (=depression) and ionic edema. It is an evolutionarily conserved phenomenon that has been documented in various animal species, from insects to mammals, in response to various forms of acute neuronal and glial stress. In humans, SD has been recorded as a critical response to a wide range of acute neurological conditions, including cerebral ischemia, intraparenchymal hemorrhage, subarachnoid hemorrhage, subdural hematoma, moyamoya vasculopathy, traumatic brain injury, and even during the dying process of the brain during continued systemic circulation and after cardiac arrest. In intact tissue, SD is accompanied by a normal hemodynamic response, i.e. initial hyperemia, and is short-lived and harmless. In contrast, SD is long-lasting under conditions of energy deprivation and the intraneuronal changes associated with SD, such as increases in Na+ and Ca2+, can eventually lead to cell death. Accordingly, the concept of the SD continuum is important because many SDs have intermediate characteristics, as opposed to the two extremes of SD in either severely ischemic or normal tissue. The SD extreme in ischemic tissue, referred to as terminal SD, is characterized by the initial, still reversible SD component, followed by the negative ultraslow potential (NUP). The NUP is the largest bioelectrical signal ever recorded from the human brain and is thought to reflect the progressive recruitment of neurons into cell death in the wake of SDs. The same SD wave can be long-lasting and lethal at one point in the tissue and gradually turn into a short-lasting and harmless wave as it spreads through the tissue. The relationship between SD and energy deficiency is complex. For example, energy deficiency following mechanical arterial obstruction triggers SD within minutes, but SD can also develop in non-ischemic tissue and trigger vasoconstriction and ischemia as a result of an inverse hemodynamic response. SD is a biomarker of neuronal injury during neurocritical care. Simultaneously, it is a compelling yet complex therapeutic target for neuroprotection. In particular, the microcirculatory response to SD is a promising target for neuroprotective strategies.

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Spreading depolarisation (SD) waveform predicts SD-mediated localised haemodynamic responses in ischaemic brain tissue

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Introduction: Spreading depolarisations (SD) interact with cerebral microvasculature triggering blood flow responses that are dependent on underlying tissue health. SDs propagating through healthy cortical tissue trigger a vasodilatory response. However, SDs initiating and propagating through ischaemic tissue can trigger hypoperfusion, due to severe arteriole constriction coupled to the SD wavefront, potentially worsening ischaemic outcome. We applied epicortical graphene micro-transistor (gSGFET) arrays, capable of wide bandwidth electrophysiology, in anaesthetised mice. Due to their transparency, gSGFETs can simultaneously operate with laser speckle contrast imaging (LSCI) allowing direct correlation with underlying regional cerebral blood flow (rCBF) within a micron scale.

Methods: The photothrombosis and distal middle cerebral artery occlusion (dMCAo) models were used to induce focal cerebral ischaemia in mice. A 30 channel epicortical DC-coupled gSGFET array was positioned over the somatosensory cortex 0.5 mm from the area of ischaemia permitting the recording from neural tissue within a gradient of perfused tissue. LSCI was employed providing monitoring of rCBF directly below up to 30 recording transistors. SD and haemodynamic responses were monitored.

Results: Perfusion deficit severity was a primary contributor to SD duration and waveform. SDs recorded from cortical tissue with a 50-60% perfusion SDs were 135±20.93 seconds in duration and double peaked. The coupled haemodynamics would be vasoconstriction or severe biphasic responses. SDs recorded from cortical tissue with a 70-80% perfusion SDs were shorter (110±35.25 s) and characterised both single and double peaked waveforms. The coupled haemodynamic response would be mostly biphasic. SDs recorded from cortical tissue with 80-90% perfusion SDs were varied (67.99±36.71 s) and characterised single peaked waveforms. The coupled haemodynamic response would biphasic and vasodilation.

Conclusions: SD interaction with cortical microvasculature may contribute to the expansion of ischaemic lesions when propagating through metabolically compromised tissue.

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Ketamine inhibits the inverse haemodynamic response to spreading depolarisation in early focal cerebral ischemia

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Introduction: The N-methyl-D-aspartate receptor (NMDAR) can be involved in spreading depolarisation (SD) initiation, propagation and duration. The therapeutic administration of ketamine, an NMDAR antagonist, has emerged as a promising treatment to block or modulate SDs in pre-clinical and clinical studies. Modulation of SDs, propagating through ischaemic tissue, could also directly modulate the SD triggered microvascular inverse responses coupled to the SD wavefront.

Methods: Induction of focal cerebral ischaemia was conducted using the photothrombotic model in anaesthesised mice. A 30 channel epicortical DC-coupled gSGFET was positioned over the somatosensory cortex. The area of ischaemia was formed 0.5 mm from the gSGFET permitting the recording from neural tissue within a gradient of perfusion deficits. LSCI was employed providing direct monitoring of rCBF directly below up to 30 recording transistors. Intraperitoneal ketamine (15 mg/kg) was administered after ischaemic induction. SD and haemodynamic responses were monitored for 2 hours.

Results: Ketamine modulated SD duration and waveform in this work, rather than blocking SD initiation. SDs recorded from cortical tissue with a 50-60% perfusion were significantly shorter (34.89±27.83) than saline injected animals (135.29±20.93) characterised a single peak waveform. This was consistent throughout all levels of ischaemic tissue, including moderately perfused tissue with a perfusion level of 70-80% (saline group 110.63±35.25, ketamine group 39.55±24.20). In the ketamine injected animals, the SD induced microvasculature responses were predominantly vasodilatory, rather than inverse haemodynamic waveforms, suggesting ketamine inhibition of inverse perfusion responses.

Conclusions: Ketamine can modulate both SDs and SD induced haemodynamic responses in ischaemic tissue. The neuroprotective effect of ketamine may result from its ability to inhibit the inverse haemodynamic response to SD in at-risk tissue.

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Effect of SIN-1 on the SD-initiated negative ultraslow potential and the no-reflow phenomenon during forebrain ischemia and reperfusion in rats

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Background: 75% of acute stroke patients have elevated blood pressure on admission. In randomized stroke trials, the indirect NO-donor glyceryl trinitrate (GTN) lowered mean arterial pressure (MAP) but improved neither regional cerebral blood flow (rCBF) nor functional outcomes. One probable reason is that the microcirculation, unlike large arteries, cannot bioactivate GTN. In contrast, the direct NO-donor SIN-1 does not require bioactivation and should therefore also release NO in small vessels, where it supports eNOS-derived NO in maintaining rCBF in the hypotensive portion of the autoregulatory curve. In a meta-analysis, SIN-1 reduced infarct volume in transient ischemia models, but rCBF data were lacking.

Method: Here we investigated SIN-1's effects during a 60-minute bilateral common carotidartery occlusion (BCCAO) and reperfusion in 48 12-14-week-old Wistar-Kyoto controls (WKY) and 48 stroke-prone spontaneously hypertensive rats (SHRsp). We calculated the difference between the median values of each variable in the last 5 minutes of BCCAO and baseline and compared them between treatment (SIN-1/control) and strain (SHRsp/WKY) using two-way ANOVA. We proceeded in the same way regarding reperfusion.

Result: SIN-1 lowered MAP in both SHRsp ($P \leq 0.001$) and WKY ($P \leq 0.001$). Across SHRsp and WKY, we found P values ≤0.05 for SIN-1 effects on rCBF ($P \leq 0.001$), spontaneous brain activity ($P=0.002$), extracellular potassium amplitude ($P=0.009$) and area under the curve ($P=0.025$), direct current (DC)-amplitude ($P=0.028$) and cumulative DC-shift duration ($P=0.001$), consistent with milder ischemia and less deviation from physiological parenchymal homeostasis in SIN-1-treated animals. For reperfusion, we found P values ≤0.05 for SIN-1 effects on MAP ($P \leq 0.001$), no-reflow area ($P=0.002$), rCBF ($P \leq 0.001$), spontaneous brain activity ($P \leq 0.001$), extracellular potassium ($P=0.008$), and DC potential ($P=0.022$), consistent with less no-reflow in SIN-1-treated animals.

Conclusions: SIN-1 emerges as a promising antihypertensive in acute stroke because it increases rCBF in ischemic tissue and reduces no-reflow.

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Improved noise reduction in dynamic vessel analysis using heartbeat filtering and RPCA

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Background/Aims: Dynamic Vessel Analyzer (DVA) measures retinal vascular reactivity by tracking micrometric diameter changes in retinal vessels through fundus video recordings. However, the resulting multichannel time-series data is prone to noise, which is inadequately addressed by current preprocessing methods. Existing techniques lack standardization and may fail to capture the full dilation profile and vascular parameters. This study proposes a preprocessing pipeline designed to improve noise removal and enhance the accuracy of DVA-derived features.

Methods: We introduced a preprocessing algorithm that first applies heartbeat filtering to identify and remove oscillations at and above the individual heartbeat frequency, eliminating high-frequency noise while preserving the vascular response. Next, we performed Robust Principal Component Analysis (RPCA) separately on arterial and venous locus-based time series to extract the underlying dilation-constriction profile while further suppressing noise and artifacts. The proposed method was evaluated on DVA recordings acquired using the Imedos Protocol, comparing 11 dialysis patients and 34 healthy controls. The extracted vascular parameters included Baseline Diameter Fluctuation (BDF), Maximum Dilation %, Maximum Constriction %, Time to Maximum Dilation, and Dilation Amplitude % (DA%), which were compared between groups using ANCOVA with age as a covariate.

Results: RPCA-based preprocessing produced the clearest separation between patient groups. DA% extraction in arteries was most significant with RPCA ($p = 0.0240$), compared to polynomial regression ($p = 0.045$) and median smoothing ($p = 0.056$). Similarly, BDF in arteries was best distinguished using RPCA ($p = 0.0024$), while polynomial regression ($p = 0.0068$) and median smoothing ($p = 0.018$) showed weaker significance. In veins, RPCA was the only method yielding a significant BDF result ($p = 0.040$), whereas polynomial regression ($p = 0.30$) and median smoothing ($p = 0.20$) did not show significance.

Conclusion: Current DVA preprocessing lacks standardization, limiting the reliability of extracted vascular parameters. The novel RPCA-based approach showed clearer and more consistent distinctions between healthy and dialysis patients. These findings suggest that RPCA enhances preprocessing by improving the reproducibility and accuracy of DVA-based vascular assessments.

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Neurodegeneration, microvascular dysfunction and their interaction: associations with cognitive performance – The Maastricht Study

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Purpose: There are few population-based human data on the interaction between neurodegeneration and microvascular dysfunction in the association with cognitive dysfunction. The main objective was to investigate, using prospectively collected population-based data, whether there was an interaction between retinal nerve fibre layer (RNFL) thickness, an index of neurodegeneration, and retinal venular diameter, an index of microvascular dysfunction, in the associations of RNFL thickness and retinal venular diameter with global cognitive performance, an index of cognitive function.

Methods*:* We used cross-sectional data from The Maastricht Study (n=1,785 participants, 47.8% men, mean age 59 years, and 24.5% with type 2 diabetes [oversampled by design]) and tested for an interaction between RNFL thickness and retinal venular diameter in the non-linear associations of RNFL thickness and retinal venular diameter with global cognitive performance. RNFL thickness was defined as low (lowest quartile) and high (highest three quartiles combined). Retinal venular diameter was defined as wide (highest quartile) and narrow (lowest three quartiles combined). All associations were adjusted for potential confounders (demographic variables and cardiovascular risk factors). In addition, we tested for interaction by hypertension.

Results*:* The association of RNFL thickness with global cognitive performance was modified by retinal venular diameter ($P_{interaction}$=0.02) and the association of retinal venular diameter with global cognitive performance was modified by RNFL thickness ($P_{interaction}$=0.02). After full adjustment, low versus high RNFL thickness was associated with lower global cognitive performance in individuals with, but not in individuals without, a wide retinal venular diameter (beta, [95% confidence interval] low versus high RNFL thickness, -0.26 [-0.43; -0.08] versus -0.03 [-0.14; 0.08], respectively); and wide versus narrow retinal venular diameter was associated with lower global cognitive performance in individuals with, but not in individuals without, a low RNFL thickness (-0.27 [-0.47; -0.06] versus 0.01 [-0.11; 0.12], respectively). Hypertension modified the

above associations. In stratified analyses, the above associations were stronger in individuals with, versus without, hypertension.

Conclusion*:* In summary, the present population-based study found an interaction between neurodegeneration, estimated from RNFL thickness, and microvascular dysfunction, estimated from retinal venular diameter, in the associations of RNFL thickness and retinal venular diameter with global cognitive performance. Hence, the interaction between neurodegeneration and microvascular dysfunction may contribute to the pathobiology of cognitive dysfunction.

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Retinal vessels and blood pressure progression in children

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Background: The global rise in childhood hypertension has been increasingly linked to early vascular dysfunction. However, longitudinal data on the relationship between microvascular alterations and the development of elevated blood pressure (BP) in children and adolescents remain limited.

Methods: In 2014, a cohort of 391 children aged 6 to 8 years underwent standardized BP assessments and retinal vessel analysis. Central retinal arteriolar (CRAE) and venular (CRVE) equivalent were measured using a retinal vessel analyzer. A follow-up assessment was conducted in 2018, during which 262 children were re-evaluated using the same standardized protocols.

Results: Over the four-year follow-up, both systolic and diastolic BP increased significantly (Δ 3.965±8.25 mmHg and Δ 1.733±7.63 mmHg, respectively), while CRAE decreased (Δ −6.325±8.55 µm), with no significant changes observed in CRVE (Δ −0.163±7.94 µm). Notably, children with narrower CRAE at baseline exhibited a greater increase in systolic BP over four years (β [95% CI]: 0.78 [0.170–1.398] mmHg per 10 µm decrease, P=0.012). Conversely, elevated systolic and diastolic BP at baseline were associated with a subsequent narrowing of CRAE (β [95% CI]: −0.154 [−0.294 to −0.014] µm per 1 mmHg, P=0.031 and β [95% CI]: −0.02 [−0.344 to −0.057] µm per 1 mmHg, P=0.006, respectively). These findings suggest that retinal arteriolar narrowing is predictive of systolic BP elevation, while higher baseline BP contributes to the development of microvascular impairments.

Outlook: In an extended 8-year follow-up conducted in 2022, dynamic retinal vessel analysis was performed for the first time in a subset of 60 children aged 12 to 14 years. Preliminary findings on the association between cardiovascular risk factors, their progression, and both static and dynamic retinal vessel parameters will be presented in addition to the results reported here.

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Retinal microcirculation in patients that have recovered from acute COVID-19 infection

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Background/Aims: The present study set out to investigate whether changes in retinal oxygen metabolism and perfusion are still present in patients after they have recovered from an acute COVID-19 infection.

Methods and Material: Forty subjects participated in the present study, of which 29 had recovered from moderate to severe COVID-19 within 2 to 23 weeks before inclusion and 11 had never had COVID-19, as confirmed by antibody testing. Retinal blood flow was measured using laser speckle flowgraphy and retinal oxygen saturation as well as retinal vessel calibers were assessed with a commercially available dynamic vessel analyzer (DVA, Imedos, Germany). In addition, blood plasma samples were drawn for mass spectrometry-based multi-omics profiling, including proteomics, metabolomics and eicosadomics. The study protocol was approved by the ethics committee of the Medical University of Vienna and was conducted in compliance with the Declaration of Helsinki and the Good Clinical Practice (GCP) guidelines of the European Union.

Results: Retinal blood flow was significantly lower in patients (60.6 ± 16.0 a.u.) compared to healthy controls (76.2 ± 12.1 a.u., $p = 0.006$). Arterio-venous difference in oxygen saturation was significantly decreased in patients ($p = 0.023$ vs. healthy subjects) and the same was found for arteriovenous caliber ratio ($p = 0.021$ between groups). Molecular profiles showed a down-regulation of cell adhesion molecules, NOTCH3 and fatty acids, and suggested a bisphasic dysregulation of nitric oxide synthesis in patients that had recovered from COVID-19 infection.

Conclusion: Retinal perfusion and oxygen metabolism seems to be significantly altered in patients well beyond the acute phase of COVID-19. This is also reflected in the molecular profile of the blood plasma, indicating a down-regulation of nitric oxide-related endothelial and immunological cell functions.

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Crosstalk between two calcium activated chloride conductances in the wall of small arteries

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Background: The cation conductances in vascular smooth muscle cells (VSMC) are well-characterized. Less is known about the anion conductances. In VSMC from nearly all vascular beds two Ca^{2+}-activated Cl^- conductances are present. One conductance is cGMP-dependent while the other conductance expresses the conventional properties of Ca^{2+}-activated Cl^- currents and is cGMP independent.
In this study we have investigated how these two Ca^{2+} activated Cl^- conductances interact.

Methods: Whole-cell current-voltage relationships for the Ca^{2+}-activated Cl^- conductances were obtained in VSMC from rat small mesenteric arteries with patch clamp. Downregulation of bestrophin3 and TMEM16A was achieved with siRNA transfection in vivo and the mRNA and protein levels were quantified with PCR and Western blot, respectively. The proximity and interaction of bestrophin3 and TMEM16A were assessed with proximity ligation assay and co-immunoprecipitation in a smooth muscle cell line (A7r5).

Results: siRNA-induced downregulation of bestrophin3 inhibited the cGMP-dependent Cl^- conductance, while downregulation of TMEM16A inhibited both conductances. Downregulation of either proteins inhibited vasomotion, i.e., oscillations of tone. Downregulation of TMEM16A led to a reduction of bestrophin3 while downregulation of bestrophin3 was without effect on TMEM16A. Proximal ligation assay indicated that bestrophin3 and TMEM16A are in proximity to each other. This was confirmed by demonstration of co-immunoprecipitation of TMEM16A after pulldown with bestrophin3 and vice versa.

Conclusion: The two Ca^{2+}-activated Cl^- channel proteins, bestrophin3 and TMEM16A interact at the physical level. They also interact at the expressional level, i.e. downregulation of TMEM16A reduces the expression of bestrophin3. Finally, they interact functionally, i.e., both TMEM16A and bestrophin3 are needed for the cGMP-dependent conductance and for vasomotion in rat mesenteric small arteries.

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Dietary methylglyoxal attenuates blood pressure, microvascular dysfunction, arterial stiffness and insulin resistance in high-fat diet-induced diabetic mice

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Background/Aims: Type 2 diabetes (T2D) is associated with insulin resistance, vascular dysfunction, and increased cardiovascular risk. Methylglyoxal (MGO), a glycolysis byproduct, is often linked to vascular damage when produced endogenously. However, we previously demonstrated in cohort studies that dietary MGO has been associated with metabolic and vascular benefits. This study examines whether dietary MGO supplementation protects against hypertension, (micro)vascular dysfunction, and insulin resistance in high-fat diet (HFD)-fed mice.

Methods: Male C57BL/6 mice were fed a control diet (n=12), HFD (n=12), or HFD with 1 mM MGO in drinking water (n=12) for 13 weeks. Blood pressure was assessed non-invasively. Endothelial function was evaluated in saphenous arteries via wire myography. Plasma (s)ICAM-1 and sE-selectin levels were measured by ELISA. Microvascular perfusion was assessed using contrast-enhanced ultrasound (CEUS). Insulin sensitivity was determined via hyperinsulinemic-euglycemic clamp (HEC), while insulin-mediated microvascular recruitment (IMMR) was evaluated using CEUS-HEC. Arterial stiffness was measured by ultrasound pulse wave velocity (PWV) in the abdominal aorta.

Results: HFD increased systolic (+8%, p=0.03) and diastolic (+9%, p=0.02) blood pressure, which was prevented by MGO. Acetylcholine-induced relaxation was impaired by HFD (p=0.01), but prevented by MGO. Elevated plasma sICAM-1 and sE-selectin in the HFD group were reduced by MGO. CEUS analysis showed that MGO prevented HFD-induced microvascular perfusion impairment (p=0.01). HEC showed improved insulin sensitivity (p=0.03) in the MGO group, with an enhanced IMMR (p=0.03). MGO also prevented HFD-induced increases in PWV (p<0.001)

Conclusion: Dietary MGO supplementation counteracts key metabolic and vascular effects of a high-fat diet. By preserving endothelial function, improving microvascular perfusion, and enhancing insulin sensitivity, dietary MGO may mitigate risk factors for metabolic and cardiovascular diseases. These findings challenge the view of MGO as solely harmful and suggest a therapeutic role for dietary MGO in metabolic and cardiovascular diseases.

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Quantitive analysis of macular microvascular characteristics for diabetes and prediabetes using optical coherence tomography angiography

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Background: Diabetic retinopathy (DR) is the main microvascular complications of diabetes mellitus and it contributes to vision loss of adults. Optical coherence tomography angiography (OCTA) can identify early retinal microvascular abnormalities. To explore whether there are differences in the characteristics of macular microvascular among diabetes, prediabetes and NGT, and to detect the relationship between clinical data with OCTA indicators.

Methods: Multi-center cross-sectional study. All subjects underwent electronic questionnaire, physical examination, blood and urine sample collection and OCTA examination. Three hundred and ninety five subjects with NGT, 982 subjects with prediabetes and 1105 subjects with diabetes who were enrolled in the study. The differences of clinical data and OCTA indicators in three groups were compared, and the relationship between SOM vessel density(SOM-VD) and clinical data was investigated. The SOM-VD distribution in the pooled data was divided into quartiles, significant trends across increasing quartiles were estimated by the Cochran-Armitage trend test.

Results: Compared with the NGT group, the macular VD in the diabetes and prediabetes groups were decreased significantly (all $P < 0.05$). The thicknesses in SOM, NOM, SIM, NIM, IIM and TIM were obviously lower in diabetes subjects than in the NGT (all $P < 0.05$). SOM-VD was negatively correlated with age, WHR, SBP, UACR, HbA1c, FBG, HDL-C, BUN, hs-CRP and positively correlated with eGFR, ALT and Grip strength. In multiple stepwise linear regression analysis, there were independent correlation between age, FBG, HDL-C, Grip strength and SOM-VD. The multiple regression equation was $Y_{SOM\text{-}VD}=65.602-0.609X_{Age}-0.547X_{FBG}-1.707X_{HDL\text{-}C}+0.13X_{Grip\ strength}$. The relative risks for diabetes decreased significantly along with increasing SOM-VD quartiles.

Conclusions: The macular VD and macular thickness in diabetes and prediabetes are significantly lower than in the NGT. Decreased SOM-VD levels are associated with diabetes. SOM-VD as a potential markers of diabetes.

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Differential gene expression of normal, enlarged and arterialized capillaries in ischemic skeletal muscle of patients with chronic limb threatening ischemia

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Background: Hypoxia-induced arterialization of skeletal muscle capillaries has recently been identified as an important pathological feature that aggravates ischemic damage in chronic limb threatening ischemia (CLTI). This study applied transcriptomic approaches to characterize the mechanisms that drive capillary arterialization.

Methods: Amputation muscle samples from CLTI patients (n=21 patients x3 samples) were histologically analyzed, grouped according to microvascular changes and bulk RNA sequencing (RNA-seq) was performed. These data were used to compile a custom panel for spatial transcriptomics (n=6 patients) that was used to localize the differentially expressed transcripts prior found associated to capillary arterialization in the bulk RNA sequencing experiment.

Results: Histological analysis showed worsening myofiber atrophy and increasing fibrosis between myocytes along arterialization. Endothelial, macrophage, fibroblast and fibro-adipogenic progenitor (FAP) - associated genes were upregulated in the bulk RNA-seq data. VEGFA and NOS1 were downregulated but several arteriogenic growth factors were seen upregulated towards arterialized capillaries. In the tissue level analysis with spatial transcriptomics the differential capillary phenotypes displayed clear differences in transcript expression. In particular, the upregulation of PDGFRB in the vascular wall seemed to be associated with the arterialization process. Also, many transcripts of KDR downstream signaling cascade were upregulated and vascular mechanosensing related transcripts were present on arterialized capillaries.

Conclusion: Skeletal muscle cell type composition and capillary wall gene expression changed along the process of capillary arterialization in CLTI muscle. The technological advances in spatial transcriptomic methods allow the study of these changes in-situ in patient samples and therefore presents a perfect setting to develop approaches modulating the arterialization process.

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Engineered Vasculature – bridging human biology with translation

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Vasculature is undeniably one of the most complex mammalian networks and plays a key role in development and disease. Although animal models have been the mainstay of vascular biology research, the mismatch in animal-human translation has highlighted the need for new humanized models. Our group has developed a number of 3D perfusable microvascular models using both human primary and iPSC-derived donor cells. We have developed tissue-specific models of placenta, breast, lung and cardiac vasculature in order to investigate toxicology, sex hormones, infection and inflammation. In this talk, I will highlight some examples that highlight the importance of the microenvironment (shear flow, stromal cell composition, cytokines) in vascular development and how we can employ these in vitro systems to model vascular disease. I will also discuss the gaps in bridging these models with real-world translation – issues surrounding scale-up, population level representation, and the need for standardization.

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Vascular redox signalling und oxidative stress

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Reactive oxygen species (ROS) play an essential role in cell signaling. One of the most important sources of ROS is the family of NADPH oxidases, consisting of Nox1 through 5 and Duox1 and 2. NADPH oxidases are multicomponent enzyme complexes that serve diverse cellular functions under physiological and pathophysiological conditions. Short term ROS formation can be mediated by NADPH oxidase (Nox) 2. They mediate short term signaling. In contrast, long term processes such as cell homeostasis and differentiation depend on long lasting ROS formation. The organizing cytosolic subunit of the Nox1 complex, NoxO1, enables constitutive ROS formation and thus rather has a tonic effect on redox balance. By doing so, NoxO1 diminishes the transition of endothelial cells towards a tip cell phenotype. Accordingly, angiogenesis in the immature retina and in skeletal muscle is enhanced upon loss of NoxO1. As a consequence, NoxO1 knock out mice have a faster retina vascularization and recover faster from vessel injury. Both of which however come with the formation of instable and leaky vessels. Nox 1 and 2 signal at the plasma membrane using $\cdot O^{2-}$ and its subsequent decomposing products, while Nox4 due to its intracellular localization and direct formation of H_2O_2 can instantly oxidize specific nearby target proteins for long lasting alteration of their function. As such Nox4 can oxidize the histone deacetylase 4 (HDAC4) and therefore enables differentiation and once differentiated homeostasis of endothelial cells on epigenetic level.

The talk will cover some aspects of NADPH oxidase mediated signal transduction and (patho-)physiologic roles in the vasculature.

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Redox signalling, oxidative stress, endothelial dysfunction and cardiometabolic diseases

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Background/Aims: Reactive oxygen species (ROS) are important regulators of physiological function. We could show that the NADPH oxidase 4 (Nox4) generates low-dose hydrogen peroxide, reduces atherosclerotic plaque formation and mediates protective effects of physical activity against obesity-induced vascular dysfunction. In contrast, cardiometabolic risk factors like hypertension, obesity and hypercholesterolemia can induce oxidative stress, endothelial dysfunction and promote the pathophysiological development of cardiovascular diseases. Previous studies suggested a crosstalk between an activated renin-angiotensin-aldosterone system (RAAS), ROS and oxidized low-density lipoproteins (oxLDL) in atherosclerosis, but the underlying molecular mechanisms are not well understood.

Methods: The impact of angiotensin type 1 receptor (AT1R) blockade on oxLDL-induced superoxide anion formation and endothelial dysfunction was studied in human umbilical artery endothelial cells and aortic rings of wild-type mice by chemiluminescence and Mulvany myograph. Further assays included promoter deletion studies, EMSA, real-time PCR, confocal microscopy, Western blotting, G protein reporter assays, phospho-ERK1/2 antibodies and specific siRNAs. The data were validated in hearts of obese C57BL/6 mice and cardiac and aortic tissue of AT1a/AT1b double knockout mice in vivo.

Results: AT1R promoter activation studies upon Ang II- and oxLDL-stimulation in endothelial cells revealed that Ang II and oxLDL activate AT1R signaling through G protein Gα12/13, followed by activation of ERK1/2 MAP kinases, and transcription and translation of Oct-1, resulting in up-regulation of AT1R, LOX-1 and NOX2 expression, which could be antagonized by specific inhibitors at each step of the identified signaling cascade. AT1R blockade improved oxLDL-induced endothelial dysfunction in aortic rings of wild-type mice. Male C57BL/6 mice fed a high-fat diet exhibited upregulation of Oct-1 levels in cardiac tissues, compared to normal controls, while AT1a/AT1b double knockout mice demonstrated downregulation of Oct-1, AT1R, LOX-1, and NOX2 on mRNA and protein level in cardiac and aorta tissue, thus confirming the identified signaling cascade in vivo.

Conclusion: Physiological concentrations of hydrogen peroxide generated by Nox4 can mediate vasoprotective effects in the vessel wall. In contrast, cardiometabolic risk factors can increase oxidative stress and a novel molecular cross-talk of oxLDL with ROS signaling and the RAAS in endothelial dysfunction, atherosclerosis and clinical complications of cardiometabolic diseases.

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Interplay between inflammation and oxidation in hypertension

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Hypertension is an increasingly critical global health concern and a leading contributor to morbidity and mortality worldwide. It ranks among the most significant risk factors for the development of severe conditions, particularly cardiovascular and kidney diseases. Central to the pathogenesis of hypertension are oxidative stress and chronic inflammation, which play pivotal roles in both its onset and progression. These processes are further intensified by aging, contributing to the high prevalence of hypertension among older adults.

A major source of reactive oxygen species (ROS) in the vascular system is NADPH oxidases, enzymes widely expressed in endothelial cells, vascular smooth muscle cells, fibroblasts, perivascular adipocytes, and infiltrating leukocytes. When produced excessively, ROS can inflict extensive cellular damage. In parallel, heightened immune activity and the accumulation of leukocytes within the vasculature sustain a chronic inflammatory state. Together, these mechanisms impair vascular endothelial function, promote vascular fibrosis, and increase arterial stiffness.

Notably, oxidative stress and inflammation are tightly interconnected, each capable of amplifying the other in a vicious cycle that accelerates the progression of hypertension and its complications. Gaining deeper insight into the molecular interplay between these two processes not only enhances our understanding of hypertension’s pathophysiology but also reveals promising avenues for targeted therapy. Interventions that concurrently address oxidative stress and inflammation may improve clinical outcomes and prevent the long-term consequences of hypertension.

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Effect of vitamin D on vascular oxidative nitrative stress in a rat model of PCOS

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Background/Aims: Polycystic ovarian syndrome (PCOS) is one of the most frequent gynaecological endocrinological disorders. The exact etiology is still unknown. It can be accompanied by chronic inflammation and high levels of oxidative-nitrative stress markers, which might exacerbate the already present metabolic and hormonal imbalance. Our research group has previously investigated the impact of vitamin D deficiency in PCOS. The aim of the present study was to investigate oxidative nitrative stress levels under normal animal housing conditions and vitamin D supplementation in the context of a hyperandrogenic model of PCOS , and to examine these effects on the function of carotid arterial rings.

Methods and Material: For 8 weeks, four-week-old female Wistar rats were fed conventional rat chow (Control group) or supplemented with vitamin D (VitD suppl. group) via oral administration of Vigantol oil. Transdermal testosterone therapy generated hyperandrogenism in half of the rats (Testosterone group; Testosterone+Vit D suppl. group) (n=8 in all four groups). Carotid artery rings were isolated and paraformaldehyde fixed for immunohistochemistry labeling against endothelial nitric oxide synthase (eNOS), cyclooxygenase-2 (COX-2), 3'nitrotyrosine (NT), poly (ADP ribose) polymer (PAR), and 4'hydroxynonenal (HNE).

Results: PAR density was significantly lower in the Vitamin D suppl. group compared to the Control group. Optical density of PAR was significantly higher in the Testosterone+Vit D suppl. group compared to the vitamin D suppl. group. No significant difference was found for eNOS, COX-2, NT and HNE immunostainings.

Conclusion: Among the immunohistochemical markers affecting vascular function, neither eNOS nor COX-2 density showed significant differences, suggesting that vitamin D supplementation above standard levels has neither positive nor negative effects. The treatment did not affect NO metabolism, but significantly reduced PAR density. Vitamin D administration may have a beneficial effect on oxidative-nitrative stress levels in patients with or without PCOS.

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Plastic surgery procedures and wound edge microcirculation: Therapeutic options and their effectiveness

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Background/aims: The success of plastic surgery largely depends on the condition of wound edge microcirculation, which determines tissue oxygenation, wound healing, and the viability of grafts or free flaps. Various surgical, pharmacological, and physiotherapeutic methods can be applied to improve microcirculation, aiming to enhance tissue perfusion and reduce venous congestion.

Methods: A literature review was conducted to examine therapeutic options for improving microcirculation, including negative pressure wound therapy (NPWT), hyperbaric oxygen therapy (HBOT), medicinal leech therapy (hirudotherapy), as well as physiotherapy and compression techniques.

Results: All the examined therapeutic methods have been proven to improve microcirculation and wound healing through different mechanisms.

- **Negative Pressure Wound Therapy (NPWT):** By generating controlled negative pressure, NPWT enhances capillary perfusion, reduces interstitial oedema, and promotes angiogenesis. The mechanical forces exerted by the vacuum stimulate fibroblast proliferation and extracellular matrix deposition, accelerating granulation tissue formation and epithelialization.
- **Hyperbaric Oxygen Therapy (HBOT):** The increased oxygen partial pressure in the blood improves oxygen diffusion into hypoxic tissues, enhancing mitochondrial activity and ATP production. This promotes fibroblast and keratinocyte proliferation, increases collagen synthesis, and stimulates angiogenesis through vascular endothelial growth factor (VEGF) upregulation. Additionally, HBOT has antibacterial effects, reducing the risk of postoperative infections.
- **Medicinal Leech Therapy (Hirudotherapy):** The anticoagulant (hirudin) and vasodilatory substances in leech saliva prevent thrombosis and improve venous outflow in congested tissues. The bioactive molecules also have anti-inflammatory properties, reducing local cytokine release and oedema. This therapy is particularly beneficial in compromised free flaps or replanted tissues with venous congestion.
- **Physiotherapy and Compression Therapy:** Physical modalities such as ultrasound and electrical stimulation improve local blood circulation by inducing vasodilation and stimulating cellular metabolism. Compression therapy enhances venous return, reduces capillary leakage, and facilitates lymphatic drainage, decreasing oedema and improving oxygen supply to healing tissues.

Conclusion: Microcirculation-enhancing therapies in plastic surgery procedures can significantly contribute to reducing postoperative complications and achieving better functional and aesthetic outcomes. The combination of different treatment methods may be more effective in complex cases, particularly in improving the survival of free flaps and skin grafts. Further research should focus on optimizing these therapies and developing the best clinical protocols.

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Influence of visceral peritoneal desiccation to small bowel microcirculation in animal model

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Background/Aims: Reducing intestinal tissue desiccation is a common recommendation to decrease post-operative complications following open abdominal surgery. Although surprisingly we know few facts about the pathophysiological and surgical background of it. While the recommendations offer keeping the bowel moist, there is no data in the literature on the real effect of this or lack thereof. The goals of this study were to measure the local perfusion in the mesentery, and in the small bowel wall also during open abdominal surgical animal model.

Methods: Open abdominal surgical intervention was performed in 20 Wistar rats under general anaesthesia. In Group I (GI) after laparotomy, the abdomen remained open for 6 hours, but the abdominal organs were continuously moisturized. In Group II (GII) after laparotomy the small intestines were in ambient air for 6 hours without any moisturizing. Parallel with checking the systemic circulating parameters (blood pressure, heart rate, O_2 saturation, etc), local perfusion of mesenteric and intestinal wall was measured every hour with Laser Speckle Imaging System (RWD Life Science Co., Ltd).

Results: Our results demonstrated that desiccation decreases both the systemic blood pressure and the local intestinal wall microcirculation perfusion. Both the mesenteric and the intestinal wall perfusion decreased mildly after 5 hours of open and wet-hold bowel surface (GI). In contrast, mesenteric and wall perfusion significantly ($p>0.05$) decreased after 3 hours of open hold, without moisturizing. Moreover, these parameters decreased dramatically after 6 hours in GII.

Conclusion: It was concluded that during open surgery, bowel moisturizing has systemic and localized measurable effects on visceral macro- and microcirculation. Further studies are necessary in order to elucidate the questions that come up from this model.

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Microcirculation changes on nipple and areola complex following different plastic surgical experimental tehniques

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Background/Aims: During plastic breast surgery the areola and nipple (Areola mammae and Papilla mammaria) are frequently involved. Various surgical techniques are employed in this area, making it crucial to understand the anatomy, function, and microcirculation to optimize the procedures and enhance healing. Disturbances in microcirculation, often accompanied by edema, can lead to improper wound healing, complications, tissue damage, necrosis, and scarring. The objective of our animal studies was to model wound healing and microcirculation, evaluating the effects of different surgical techniques and external influences—such as thermal and mechanical forces, and topical treatments. The focus of this study is on factors that influence microcirculation and wound healing. Our goal is to improve the outcomes of nipple-plasty procedures in reconstructive breast surgeries. Utilizing laser tissue perfusion imaging allows us to predict tissue healing, plan appropriate interventions, and ultimately reduce complication rates. This study investigates wound healing and circulation changes following nipple and areola surgery, and evaluates how mechanical force, thermal treatment (cold and heat), and topical medicinal products impact recovery.

Methods and Material: Five different surgical interventions were performed on ten pairs of nipples of a swine. The surgeries included vertical breast lifting, nipple circumcision, subdermal preparation, Benelli mastopexy, nipple transplantation, and nipple bisection. Post-surgery, we measured tissue perfusion using the MoorO2Flo-2 Tissue Perfusion and Oxygenation Tool during the first and second weeks. We then examined the effects of thermal treatments (cold and heat), followed by the impact of mechanical force, applying a force of 40-50 N.

Results: One week after surgery the wound healing process was in the inflammation phase. Heat significantly increased circulation, while cold and mechanical forces led to a decrease. Topical creams affected wound healing and regeneration.

Conclusion: Wound healing can be influenced by chemical and physical factors at different stages to promote scarless healing.

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Effects of botulinum toxin a on microcirculation: laser doppler assessment of blood flow alterations

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Background/Aims: Botulinum toxin is widely used for its neuromodulatory effects, but its impact on microcirculation remains less explored. This study investigates whether botulinum toxin A alters local cutaneous blood flow dynamics. Microvascular changes are assessed using Laser Doppler to provide objective quantification. Our aim is to understand its potential effects on tissue perfusion and vascular regulation.

Methods and Material: Fourteen adult volunteers participated in this study, including 13 females and 1 male. Using Laser Doppler flowmetry (LDF) changes in skin perfusion were measured before, during and after 30 second temporary digital compression of the superficial temporal artery. Patients then received Botulinum toxin A (BTX-A) treatment around the glabellar and frontal areas. After 2 minutes the Laser Doppler flows were then again recorded before, during and after 30 second compression of the artery. Measurements were performed on the skin above the superficial temporal artery using Perimed 5000 Laser Doppler flowmetry for continuous perfusion monitoring.

Results: Initial mean blood flow was 79,33 +/- 19,58 perfusion unit (PU), and all signals were pulsatile. The blood flow decreased by a mean of 6% (74,58 +/- 20 PU) after botulinum toxin treatment. With compression of the artery blood flow reduced by 25,73% (mean 58,92 PU) before treatment and by 21,87% (mean 58,27 PU) after. A decrease in pulsatility was also observed. On release of digital compression of the superficial temporal artery perfusion increased by 16,29% (mean 92,25 PU) before BTX-A and by 2,88% (mean 76,73 PU) after. Pulsatile pattern also improved.

Conclusion: This study demonstrates that botulinum toxin A (BTX-A) slightly reduces baseline skin perfusion and modifies the vascular response temporary arterial occlusion. The diminished hyperemic response suggests a regulatory effect of BTX-A on microvascular dynamics. These findings enhance our understanding of BTX-A's vascular effects, with potential implications for both vascular and aesthetic medicine.

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Beneficial effects of anti-inflammatory drugs, negative pressure therapy, autologous fat grafting therapy and hyperbaric oxygen therapy on wound healing

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Background/Aims: Skin microcirculation plays a crucial role in flap survival in reconstructive plastic surgery. We aim to enhance tissue survival and wound healing by applying new therapeutic methods. The survival of the skin flaps is determined by its microcirculation in the early critical postoperative period. Improving this microcirculation can reduce the risk of flap necrosis.

Methods: We investigate the beneficial effects of anti-inflammatory drugs, negative pressure therapy, autologous fat grafting therapy and hyperbaric oxygen therapy, as they may have beneficial effects on wound healing by modifying the venous microcirculation. Microcirculation was assessed using laser Doppler measurements, which examine blood circulation and perfusion in tissues. The measurements were performed using the PeriFlux 5000 Laser Doppler device. This assessment was performed on days 1-5. The flow rate was measured in perfusion units (PU) set by the instrument, allowing for intercomparison.

Results: Antioxidant drugs increase perfusion within an hour. NPWT increases microcirculation up to 3rd post-op days. Hyperbaric oxygen therapy reduces tissue perfusion in the first hours due to higher oxygen levels but increases it in the next 24 hours. ADSC treatment elicits no significant differences in wound microcirculation in the first 5 postoperative days

Conclusion: Three methods are available to increase tissue perfusion in the early days after surgery: negative pressure therapy, hyperbaric oxygen therapy and antioxidant drugs. Autologous fat therapy may be promising in wound healing, but not by increasing tissue perfusion.

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Increased muscle mass protects against ischemic injury in the lower limb

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Obesity and associated metabolic disease are major drivers of peripheral artery disease (PAD) in the lower limb. Exercise mitigates these effects, improving or preventing PAD but the mechanisms by which exercise exerts this protection is unknown. A significant component of exercise-induced changes in limb physiology are increases in the quantity and quality of skeletal muscle. We have modeled this phenomenon by creating a mouse model in which myostatin (MSTN), the rate-limiting myokine for muscle growth, is deleted in obese, diabetic *db/db* mice. Control obese mice demonstrate poor glucose regulation, reduced endothelial function and impaired perfusion of the lower limb. Endothelial function, but not glycemic control, are rescued by deletion of super-oxide producing, largely vascular restricted, NADPH Oxidase 1 (NOX1) and pro-inflammatory Galectin-3 (GAL3). Using a surgical model of hindlimb ischemia (HLI) it is observed that deletion of MSTN increases muscle mass and mitigates the loss of hindlimb reperfusion post-HLI and capillary rarefaction in obese diabetic mice *in vivo* and normalizes angiogenesis in *ex vivo* aortic ring assays. Moreover, MSTN KO reduces fibro-fatty replacement of damaged skeletal muscle, preserving tissue regeneration, and reducing muscle loss. While deletion of NOX1 in *db/db* mice normalizes angiogenesis and protects limb reperfusion, it does not prevent fibro-fatty degeneration. On the other hand, GAL3 KO in *db/db* mice not only protects angiogenic capacity and limb reperfusion but also ameliorates fatty lesion formation, suggesting that resolution of vasculogenic deficit alone is insufficient; rather, both inflammation resolution and rescue of downstream angiogenesis are necessary for limb preservation in the context of obesity-induced vascular disease. The cell types and molecular agents mediating this preservation remain to be determined.

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Systemic microvascular endothelial dysfunction as a target for diagnosis and treatment of sex-specific cardiac disease

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Background/Aims: Cardiovascular diseases are the leading cause of death in women, including heart failure with a preserved ejection fraction (HFpEF), and are strongly associated with (pre)diabetes. In humans and mice with prediabetes and type 2 diabetes, we previously showed that microvascular dysfunction associates with obesity and type 2 diabetes and is triggered by inflammation of perivascular adipose tissue. In HFpEF, microvascular function predicts disease progression. Here, microvascular dysfunction will be discussed as a risk factor and treatment target in HFpEF.

Methods and Material: all necessary ethical approvals for our research were obtained prior to the studies. Microvascular function was studied using laser speckle contrast analysis (LASCA) in human subjects with type 2 diabetes and related to risk of heart failure with preserved ejection fraction. In patients with HFpEF, we evaluated the efficacy of empagliflozin and iron supplementation on microvascular function, two interventions with proven efficacy in improving symptoms burden in HFpEF.

Results: In the Hoorn Diabetes Care System Cohort, we found in 152 patients that systemic dysfunction of microvascular endothelium, particularly insulin-stimulated vasoreactivity, associates with risk of future HFpEF development in women but not in men. In an open-label study in 22 obese patients with HFpEF, we found that three months treatment with empagliflozin enhanced insulin-stimulated vasoreactivity without major effects on body weight, insulin levels of blood pressure. This effect was strongly related to effects of empagliflozin on adiponectin and endothelin-1. In contrast, empagliflozin treatment attenuated acetylcholine-stimulated vasodilatation. In contrast to the effect of empagliflozin, iron supplementation improved acetylcholine-stimulated vasodilation in HFpEF patients but improved insulin-stimulated vasodilatation only in women.

Conclusion: Systemic microvascular dysfunction, particularly insulin-stimulated vasoreactivity, is strongly associated with the development of HFpEF. Empagliflozin and iron supplementation provide new tools to improve systemic microvascular endothelial function, and microvascular function provides a new druggable target for prevention and management of HFpEF.

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Role of PBK and metabolic reprogramming in pulmonary vascular remodeling

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Pulmonary arterial hypertension (PAH) is a rare and debilitating condition with a low survival rate and limited therapeutic tools. Elevated pulmonary arterial pressure arises from inappropriate remodeling of pulmonary blood vessels that is due, in part, to phenotypic changes in pulmonary arterial smooth muscle cells (PASMC). Similar to cancer, vascular remodeling in PAH has characteristics that are hyperproliferative and anti-apoptotic. We recently found that the gene, PDZ-Binding Kinase (PBK) is highly upregulated in human and experimental pulmonary hypertension. PBK is a serine/threonine kinase that has important roles in mitosis and cell proliferation. In normal tissues, PBK levels are typically very low, however expression levels are significantly increased in highly proliferative tissues such as cancer. We found that PBK was upregulated in the medial layer of PA, overlapping with markers of smooth muscle cells in experimental mouse and rat models of pulmonary hypertension (PH) as well as human PAH. In gain of function approaches, active forms of PBK increased pulmonary artery smooth muscle cell (PASMC) proliferation, whereas silencing, dominant negative PBK and pharmacological inhibitors of PBK all reduced proliferation. Pharmacological inhibitors of PBK were effective in PH reversal strategies in both mouse and rat models providing translational significance. In a complementary genetic approach, PBK was knocked out in rats using CRISPR/Cas9-editing and loss of PBK in rats prevented the development of multiple indices of PAH. Mechanistically, the ability of PBK to promote proliferation and metabolic reprogramming was associated with a major increase in glycolysis. In summary, PBK is a newly described target for PAH that is upregulated in proliferating PASMC where it contributes to proliferation and vascular remodeling.

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Venous obstruction as the primary cause of tubular injury in ischemic Acute kidney injury.

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Acute kidney injury (AKI) is a major clinical problem. Ischemic AKI is the most common form of AKI. Currently there are no treatments to prevent ischemic AKI and a better understanding of the pathogenesis of ischemic AKI is needed.

The histopathology of ischemic AKI is remarkably similar across a broad range of etiologies. This includes a swollen pale cortex and red blood cell (RBC) congested outer-medulla. Tubular injury is often observed in close proximity to the vasculature and is most prominent in the cortical medullary junction around the area of RBC trapping.

While traditionally, tubular injury in ischemic AKI has been thought to be primarily due to hypoxia, we have recently reported that toxic injury associated with the extravasation of blood proteins from the RBC congested outer-medulla vasculature may be the predominant cause of injury.

Recent work demonstrates that there is significant extravasation of blood proteins from the outer-medullary capillaries within minutes of reperfusion of the kidney and that this is associated with toxic tubular injury. We demonstrate that RBC trapping and extravasation of blood proteins occurs secondary to occlusion of the large renal veins in early reperfusion.

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Always along the microvascular path … and sitting in the neutrophil cockpit

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The observation and functional peculiarities of the microvasculature using intravital imaging have accompanied my scientific life since the beginning of my medical doctoral studies. In this collaborative effort, we succeeded in demonstrating, under in vivo conditions and using a two-micropipette injection system, that the removal of the endothelial glycocalyx in microvascular networks of the rat mesentery leads to a significant drop in blood flow resistance. Using my skills in intravital imaging, I expanded my field of interest and investigated the behavior of neutrophils in inflamed microvessels, which soon became my main research focus. Initially, I concentrated, in a first line of studies, on selectin-dependent neutrophil rolling and the influence of posttranslational glycosylation (sialylation) on this process. Interestingly, these studies revealed that, besides rolling, firm arrest and extravasation of neutrophils also depend on proper glycosylation, involving additional adhesion-relevant molecules such as chemokine receptors.

Influenced by my clinical work in neonatal medicine, I also started a second line of research, for which we developed an in vivo model of inflammation in the mouse yolk sac and embryo. Combined with a sophisticated in vitro microflow chamber system we had established, we were able to show that neutrophil recruitment is ontogenetically regulated, both in mice and humans. Subsequent work using an omics approach enabled us to further elucidate the molecular players regulating neutrophil function during mouse fetal life and human development, highlighting also an important role for the non-canonical NF-κB pathway in this process.

In a third line of studies, my lab focuses on the DAMP molecule and Ca^{2+}-binding protein S100A8/A9, the most abundant cytosolic protein in neutrophils. Here, we recently uncovered an E-selectin-mediated S100A8/A9 release mechanism, which depends on the activation of the NLRP3 inflammasome and subsequent formation of Gasdermin D pores, through which S100A8/A9 is released into the extracellular space, where it can act as a DAMP molecule.

In my talk, I will present work from these three lines of research and also introduce key mentors, collaborators and friends I worked together with over the years.

Supported by German Research Foundation (DFG) Collaborative Grants TRR359 Perinatal Immunology and TRR332 Neutrophil. More information at https://orcid.org/0000-0002-7689-3613.

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The influence of oversized plain old balloon angioplasty on endothelial injury in swine arteries

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Background/Aims: The balloon-to-artery ratio (BTAR) is the inflation balloon diameter relative to the artery diameter that indicates the ratio of arterial stretching. Oversized BTARs (≥1.3:1) have been linked to arterial overstretching and associated with complications such as arterial dissection and intima hyperplasia. We aimed to determine the effect of plain old balloon angioplasty (POBA) BTAR on endothelial integrity in an *ex vivo* swine artery perfusion model.

Methods and Material: Thirty *ex vivo* swine (Yorkshire pig) external iliac arterial pairs were collected from an abattoir and equally allocated to 3 treatment groups: BTAR 1.3:1, BTAR 1.1:1, or BTAR 1:1 (n = 60 arteries). The artery of one side was selected for a POBA treatment using the allocated BTAR, and the contralateral artery was used as a perfused control without angioplasty. Arteries of the treatment and control groups were perfused within the *ex vivo* flow model with porcine blood for 9 minutes, including 3 minutes of POBA in the treatment group. Endothelial integrity was assessed by calculating the presence of endothelial denudation (i.e., percentage of macroscopic denudation using Evans blue), and histopathologic analyses on formalin-fixed, paraffin-embedded tissue sections stained for erythroblast transformation-specific–related gene (ERG) for detection of endothelial cell nuclei and Dolichos biflorus agglutinin (DBA) lectin for detection of endothelial glycocalyx.

Results: Macroscopic endothelial denudation assessment with Evans blue showed a 55% increase in endothelial denudation in the 1.3:1 BTAR group compared with the perfused controls ($p<0.01$) and a 42% increase compared with the 1.1:1 and 1:1 BTAR groups (both $p<0.01$). ERG staining showed a 24% reduction in the number of endothelial nuclei for the 1.3:1 BTAR and 1.1:1 BTAR groups compared with their controls ($p=0.01$ and $p=0.03$, respectively). No significant differences were observed in the binding of DBA to endothelial glycocalyx between any of the ballooned experimental groups or their controls.

Conclusion: In this *ex vivo* study, oversizing of the balloon in a ratio of 1.3:1 compared with the artery diameter induces significant endothelial injury and may be related to a higher risk of complications developing in peripheral arterial disease patients undergoing POBA.

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The dual role of red blood cells in cardioprotection: reduction of myocardial infarct size and coronary no-reflow

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Background/Aim: For decades, cardioprotection studies have focused primarily on cardiomyocytes, i.e. infarct size reduction. However, also the extent of microvascular injury during acute myocardial infarction determines the patient's prognosis. Remote ischemic conditioning (RIC), induced by brief cycles of ischemia/reperfusion (I/R) in tissues remote from the heart, induces the release of humoral cardioprotective factors. RIC's cardioprotection is transferable from RIC-conditioned donors to ex vivo naive heart preparations via plasma-dialysates or platelets. Previous studies demonstrated that the infusion of red blood cells (RBCs) into ex vivo isolated heart preparations reduced infarct size *per se*. Here, we aim to address RBC's role in cardioprotection by RIC and not only focus on infarct size but also on the microvascular injury.

Methods: Healthy volunteers (3 females, 10 males; 27±5 years) underwent RIC via three cycles of 5/5 minute upper-arm I/R (200 mmHg). Venous blood samples were collected before and 60 minutes after RIC, blood plasma and RBCs were separated by centrifugation. Plasma-dialysates (12–14 kDa cutoff, dialysis ratio 1:10 against buffer) and washed RBCs (5×10^4/mL) were infused into isolated perfused rat hearts for 8 minutes, followed by a 2-minute washout period before 30-minute left anterior descending coronary occlusion followed by 120-minute reperfusion. Saline infusion served as control. The area at risk was demarcated by patent blue, microvascular injury (no-reflow) by thioflavin-S, and infarct size by triphenyl-tetrazolium-chloride. No-reflow and infarct size were quantified and calculated as percent of area at risk.

Results: Infarct size/no-reflow was 45±9%/9±4% with saline infusion and 43±5%/9±3% with plasma-dialysate infusion before RIC. Plasma-dialysate after RIC reduced infarct size to 34±8% but had no effect on no-reflow (9±4%). In comparison to saline and plasma-dialysate, RBC infusion before RIC reduced infarct size to 34±11% but did not affect no-reflow (12±6%). However, RBC infusion after RIC reduced infarct size further to 20±9% and also decreased no-reflow to 5±2% ($p<0.05$ versus before).

Conclusion: In line with prior studies, RBCs reduced infarct size *per se* in isolated perfused hearts. However, RIC enhanced RBC's cardioprotective potential and extended it to the coronary microcirculation. Thus, RBCs may serve as a unique novel target for delivering protection to the microcirculation.

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Role of matrix metalloproteinases and biglycan in microvascular obstruction during ischemic conditioning in myocardial ischemia-reperfusion injury

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Background: Myocardial ischemia-reperfusion injury (MIRI) leads to microvascular obstruction (MVO), which is a critical determinant of post-ischemic myocardial dysfunction. Matrix metalloproteinases (MMP-2 and MMP-9) are activated during MIRI, contributing to the degradation of cardiac contractile proteins and exacerbating MVO. In previous investigation, ischemic postconditioning (IPoC) failed to decrease myocardial necrosis in porcine model of acute myocardial infarction, however, it significantly reduced the severity of MVO. Additionally, biglycan (BGN), a small leucine-rich proteoglycan, has been identified as a potential cardioprotective factor in ischemic conditioning. Here, we hypothesized that MMP and BGN signaling play a key role in modulating MVO during ischemic conditioning.

Methods: In this study, AMI was induced in young domestic pigs (25-35 kg) using balloon catheter occlusion of the left descending coronary artery for 90 minutes. Ischemic postconditioning was performed with 6×30 s ischemia-reperfusion cycles after 90 minutes of occlusion. For reperfusion, 3 hours or 3 days of time period was applied. A sham-operated group served as a control. Plasma and left ventricular tissue was harvested to measure MMP-2, MMP-9 activities, BGN levels, and miRNA regulation associated with MMPs and BGN. A reverse target-microRNA prediction was performed to identify human microRNAs that potentially regulate MMP and BGN expression.

Results: MMP-2 and MMP-9 activities significantly increased in the IPoC group at 3 hours of reperfusion compared to ischemic controls, with no further changes at 3 days. In contrast, BGN levels were significantly decreased in the IPoC group after 3 hours of reperfusion, which were restored by 3 days. Additionally, we identified 9 miRNAs that were counter-regulated by ischemic postconditioning and potentially regulate the cardioprotective effects of MMPs and BGN in the context of MIRI.

Conclusions: Ischemic postconditioning reduces MVO and modifies MMP and BGN signaling, particularly during early reperfusion. The early decrease in BGN levels, alongside increased MMP activity, may contribute to the beneficial effects of IPoC on MVO. The identification of cardioprotective miRNAs that regulate MMP and BGN signaling opens new avenues for targeted therapies aimed at mitigating microvascular obstruction in acute myocardial infarction.

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Blood pressure lowering by sphingosine kinase 2 inhibition improves outcome after myocardial infarction

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Background/Aims: Hypertension remains a major cardiovascular risk factor despite stringent blood pressure (BP) control due to treatment failures. Targeting immune mechanisms may provide new therapies to lower BP and improve post-myocardial infarction (MI) cardiac repair, particularly in hypertension-associated heart failure (HF) patients. This study investigates whether modulation of sphingosine-1-phosphate (S1P) signaling can serve as an antihypertensive strategy and, in turn, reduce post-MI damage and slow HF progression.

Introduction: S1P, generated by sphingosine kinase 2 (SphK2), has been implicated in hypertension development and regulation of immune cell function. We have shown that pharmacological SphK2 inhibition has therapeutic potential for lowering BP by modulating immune cell responses relevant to hypertension. This study examines whether SphK2 inhibition provides antihypertensive benefits and, as a consequence, improves outcomes following MI.

Methods and Material: Male C57BL6/N mice were subjected to Angiotensin II (AngII) or saline infusion for 6-weeks. After 4-weeks of AngII-treatment, mice received subcutaneous SphK2-inhibitor (ABC294640) or vehicle treatment for 2-consecutive-weeks. Thereafter, mice were subjected to experimental MI by permanent left anterior descending coronary artery ligation. BP was monitored using tail-cuff plethysmography. Cardiac function was longitudinally assessed using magnetic resonance imaging. To quantify the effect of the inhibitor, hearts were dissected into right (RV) and left ventricles (LV) for gene expression and mass spectrometry.

Results: Prior to MI, SphK2 inhibition lowered BP independently of vascular function and plasma S1P levels. Neurohumoral compensation, evidenced by increases in heart rate and BP between 9-to-12-weeks post-MI, was absent in SphK2-inibitor-treated mice. Post-MI, a lowering of plasma S1P levels was observed in hypertensive mice. Stroke volume and end-diastolic volume inversely correlated with plasma S1P. Left ventricular ejection fraction was significantly lower in hypertensive compared to normotensive and SphK2-inhibitor-treated mice at different time points post-MI. In addition, tissue S1P concentrations at 12-weeks post-MI were lower in LV compared to RV only in both the normotensive and hypertensive groups. Moreover, SphK2 inhibition attenuated MI-associated natriuretic peptide b and S1P receptor1 upregulation.

Conclusion: Hypertension worsens post-MI outcomes and accelerates HF progression. SphK2 inhibition lowers BP, improves post-MI recovery, and may represent a promising therapeutic target for both hypertension management and cardiovascular protection.

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Human recombinant neuregulin-1β reduces the plasma levels of the tryptophan and kynurenine pathway metabolites in uremic cardiomyopathy in rats

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Aim: Here, we aimed to investigate the effects of rhNRG-1β on the plasma levels of tryptophane and kynurenine pathway metabolites in chronic kidney disease.

Introduction: The tryptophan and kynurenine pathway metabolites were shown to be associated with inflammation and a higher risk of heart failure. Low-grade microvascular inflammation and altered expression of endothelium-derived neuregulin-1β are known contributors to diastolic dysfunction in heart failure. We have previously shown that human recombinant neuregulin-1β (rhNRG-1β) ameliorated the severity of chronic kidney disease (CKD) and left ventricular hypertrophy, fibrosis, diastolic dysfunction, and the overexpression of inflammatory markers in uremic cardiomyopathy in rats.

Materials and methods: Male Wistar rats were randomized into 3 groups: 1) sham-operated group treated with iv. Saline (1 mL/kg for 10 days), 2) CKD induced by 5/6 nephrectomy group treated with iv. saline and 3) rhNRG-1-treated (iv. 10 µg/kg/day for 10 days) CKD group.

Results: Ten weeks after the operations, rhNRG-1β significantly reduced the serum carbamide and creatinine levels and increased the creatinine clearance in CKD. In addition, both the left ventricular hypertrophy and diastolic dysfunction were significantly improved by the rhNRG-1β treatment in uremic cardiomyopathy. Moreover, the plasma level of tryptophan was decreased, and the levels of several kynurenine pathway elements, including kynurenine, kynurenic acid, 3-hydroxy-kynurenine, and anthranilic acid, were significantly increased in response to CKD. The rhNRG-1β treatment significantly ameliorated these plasma level changes of tryptophan and kynurenine pathway metabolites in CKD.

Conclusion: In conclusion, the elevated plasma levels of the kynurenine pathway metabolites might be associated with the development of uremic cardiomyopathy. The rhNRG-1 treatment improved the severity of both uremic cardiomyopathy and renal dysfunction and parallelly reduced the plasma levels of the kynurenine pathway metabolites.

Ethics approval: This investigation conformed to the EU Directive 2010/63/EU and was approved by the regional Animal Research Ethics Committee of Csongrád County (XV.2598/2020, date of approval: 18 September 2020) and the University of Szeged in Hungary. All institutional and national guidelines for the care and use of laboratory animals were followed.

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Investigating the proarrhythmic effect of action potential alternans in human heart failure

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Background/Aims: Heart failure (HF) is a complex, progressive disease characterized by structural and electrical remodeling of the heart, significantly increasing the likelihood of ventricular fibrillation (VF). However, before VF develops, a potentially reversible process, known as alternans, may occur. Action potential (AP) alternans refers to the beat-to-beat oscillation of AP duration (long-short), leading to repolarization inhomogeneity.

Since no specific pharmacological intervention exists to prevent alternans, our study investigated the effect of the selective Na^+/Ca^{2+} exchanger (NCX) inhibitor ORM-10962 on alternans formation in both HF and healthy human hearts.

Methods: For our experiments, we used tissue samples from human HF and healthy hearts. HF samples were provided by the Városmajori Heart and Vascular Center, while healthy tissues were obtained from human donor hearts. APs were recorded using the conventional microelectrode technique.

Results: In HF samples, AP duration (APD) alternans were significantly larger than those observed in healthy hearts. In several cases, substantial amplitude alternans were detected in left ventricular HF hearts, primarily at higher pacing frequencies, eventually progressing to a 2:1 conduction block. Treatment with 1 μM ORM-10962 shortened the APD in HF samples, reduced the magnitude of APD alternans, and shifted the onset of amplitude alternans and conduction block to higher frequencies.

Conclusion: Electrical remodeling in human HF markedly enhances APD alternans, which may create an arrhythmogenic substrate. The simultaneous presence of amplitude and APD alternans can result in a particularly severe arrhythmogenic state, highlighting a potential link between alternans and VF development. Selective NCX inhibition shortens APD, thereby attenuating alternans and shifting their occurrence to higher frequencies, a process that may also involve additional Ca^{2+}-dependent mechanisms.

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Investigation of the effects of the phosphodiesterase-5 inhibitor tadalafil in a rat model of uremic cardiomyopathy

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Background: Chronic kidney disease (CKD) is a public health problem affecting 1 out of 10 people worldwide. Its common complication is uremic cardiomyopathy characterized by diastolic dysfunction (DD) and left ventricular hypertrophy (LVH) with fibrosis. The phosphodiesterase-5 inhibitor tadalafil was reported to inhibit cardiac remodeling in chronic heart failure of different etiologies. Here, our aim was to investigate the effects of tadalafil on the development of uremic cardiomyopathy in a rat model.

Methods: CKD was induced by 5/6 nephrectomy in male Wistar rats (350-400 g), while the control group underwent sham operation. Animals were divided into 4 groups treated via *per os gavage* from week 5: i) tap water-treated (1 mL/kg/day) sham-operated, ii) tap water-treated (1 mL/kg/day) CKD, and iii) a lower dose of tadalafil-treated (0.5 mg/kg/day) CKD, and iv) a higher dose of tadalafil-treated (1 mg/kg/day) CKD group. At week 13, laboratory tests for renal functional parameters and blood pressure measurements were performed, cardiac function and morphology were assessed by echocardiography, and the left ventricular expression of selected genes was measured by RT-qPCR.

Results: The lower dose of tadalafil significantly attenuated several echocardiographic signs of LVH and DD, whereas the higher dose of tadalafil did not affect these parameters compared to the CKD-only group. The lower dose of tadalafil did not influence the laboratory parameters of CKD and the blood pressure values, whereas the higher dose of tadalafil further worsened these parameters in CKD. Moreover, the B-type natriuretic peptide (*Nppb)* was significantly overexpressed, and the connective tissue growth factor (*Ctgf)* expression showed an increasing tendency in response to the higher dose of tadalafil compared to the CKD-only group.

Conclusion: In conclusion, the lower dose of tadalafil may attenuate the severity of uremic cardiomyopathy, whereas the higher dose treatment may worsen renal function and increase blood pressure in CKD.

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Alteration of microvascular reactivity in patients with hypertension and chronic kidney disease is related to increased oxidative stress

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Background/Aims: In its insufficiently researched etiopathogenesis, chronic kidney disease (CKD) has a recently established endothelial dysfunction and increased oxidative stress. However, the link between the two conditions has not yet been examined.

Introduction: Present pilot study aimed to determine if there were alterations in peripheral microcirculation reactivity and oxidative—antioxidative status in patients with hypertension and CKD.

Methods and Material: This cross-sectional study included 27 age and sex matched participants (non-smokers) of both sexes: hypertensive (N= 10), CKD (N=7) and controls (N=7). All patients took their medications. In addition to anthropometric, biochemical and arterial blood pressure measurements, microvascular responses of peripheral skin microcirculation to occlusion (PORH), and iontophoresis of acetylcholine and sodium nitroprusside (SNP) were assessed by laser Doppler flowmetry. Intracellular oxidative stress was measured using the BD FACSCanto II cytometer, with DCF-DA to determine H_2O_2, $ONOO^-$, and DHE to determine $O_2{\cdot}^-$ levels in peripheral blood mononuclear cells (PBMCs), while activity of antioxidative enzymes was determined by spectrophotometry. Study was approved by Ethical committee (#2158-61-46-24-121). Participants gave written informed consent (ClinicalTrials.gov.Identifier: NCT06316284)

Results: CKD patients had significantly bigger waist circumference and waist-to-hip ratio suggesting obesity, higher values of serum urea and urates and higher systolic blood pressure than controls; higher urine albumins, total proteins and lower Na^+ excretion compared to all other groups. Acetylcholine-induced dilation was significantly lower in CKD compared to controls, while SNP-induced dilation and PORH were similar among groups. PBMCs' production of H_2O_2/$ONOO^-$ was significantly higher in hypertensive compared to controls. Superoxide production was significantly lower in hypertensive compared to other groups. H_2O_2 production was significantly higher compared to controls and superoxide lower in CKD compared to controls and hypertensive patients. Activity of serum catalase and glutathione peroxidase (GPx) was similar among groups. SOD activity was decreased in hypertensive, but increased in CKD patients. Positive correlation of GPx and H_2O_2 production was present in CKD patients (r=0.96, p=0.009).

Conclusion: CKD patients exhibit impaired endothelium-dependent vasodilation in microcirculation and increased production of oxidative stress markers accompanied by impaired activation of antioxidative systems.

Funded by Faculty of Medicine Osijek institutional grants: IP18- MEFOS-2024 and IP20- MEFOS-2025 (PI I. Drenjančević).

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Investigation of the sex-based differences in the severity of doxorubicin-induced chronic cardiotoxicity in a rat model

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Background/Aims: Doxorubicin (DOXO) is a widely used chemotherapeutic agent. The development of chronic cardiotoxicity might limit its clinical application. The severity of DOXO-induced chronic cardiotoxicity might also be influenced by female sex. In our present study, we aimed to investigate the potential effects of sex-based differences on the development of DOXO-induced chronic cardiotoxicity in a rat model.

Methods: Therefore, male and female Wistar rats (300-400 g) were divided into 2-2 groups: control treated with physiological saline (ip. 1 ml/kg in 6 cycles for 2 weeks) and DOXO (ip. 1 mg/kg in 6 cycles for 2 weeks) groups. Then, the animals were followed up for 13 weeks. At week 12, echocardiography was performed to assess cardiac function and morphology. At week 13, histopathological examinations of isolated heart samples were performed to assess fibrosis, and RT-qPCR was used to assess the left ventricular expression of marker genes associated with myocardial stretch and fibrosis.

Results: Echocardiography revealed diastolic dysfunction and a significant thinning of the left ventricular walls after DOXO compared to the sex-matched controls. However, only males showed a significant increase in the left ventricular end-systolic diameter and a marked decrease in ejection fraction. The degree of fibrosis was similar in both sexes.

Conclusion: In response to DOXO, chronic cardiotoxicity characterized by wall thinning, diastolic dysfunction, and fibrosis developed in both sexes, but marked systolic dysfunction was only observed in males using the present dose of DOXO. Further histopathologic and molecular measurements are needed to better characterize the sex-based differences in our DOXO-induced cardiotoxicity model. Furthermore, our model may be suitable for testing new agents to attenuate DOXO-induced chronic cardiotoxicity in both sexes.

Key words: *doxorubicin, cardiotoxicity, fibrosis*

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Investigation of the potential sex-based differences in the development of trastuzumab-induced chronic cardiotoxicity in a rat model

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Backround/Aims: Human epidermal growth factor receptor 2 (HER2) overexpression occurs in one-third of breast and gastro-esophageal cancers. Although the monoclonal antibody against HER2, trastuzumab (TZB), significantly increases the survival of cancer patients, cardiac damage as its side effect is a common complication and causes premature death. Here, we investigated the potential sex-based differences in the development of trastuzumab-induced chronic cardiotoxicity.

Methods: Male and female Wistar rats (300-400 g) were divided into 2-2 groups: control treated with saline (ip. 6x1 mL/kg in 2 weeks) and TZB (ip. 2 mg/kg, then 5x1 mg/kg in 2 weeks) groups. The animals were followed up for 20 weeks. At week 19, echocardiography was performed to assess cardiac function and morphology. Then, picrosirius red and fast green staining of isolated heart samples was performed at week 20 to measure the extent of fibrosis, and RT-qPCR was used to assess the left ventricular expression of marker genes associated with cardiac remodeling.

Results: There was no detectable change in the body, left ventricular and lung weights in response to TZB compared to their sex-matched control group. Our echocardiographic results showed the development of diastolic dysfunction characterized by a significant decrease in e’ and a marked increase in E/e' in the TZB-treated groups in both sexes. However, there was no significant difference in the ejection fraction between the groups. The left ventricular collagen content and the Col1a1 and Col3a1 expressions were significantly increased in both sexes compared to the sex-matched control groups.

Conclusion: In conclusion, TZB induced diastolic dysfunction and cardiac fibrosis in both sexes; however, marked systolic dysfunction failed to develop in our present rat model. Further histopathologic and molecular measurements are needed to better characterize our TZB-induced cardiotoxicity model in both sexes.

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Changes in tryptophan metabolism in paclitaxel-induced chronic cardiotoxicity in a rat model

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Background/Aims: Although the commonly used chemotherapeutic agent paclitaxel (PTX) significantly increases survival in lung and breast cancer patients, it can also have the side effect of chronic cardiotoxicity, the pathomechanism of which is poorly understood. The aim of this project was to set up a model of PTX-induced chronic cardiotoxicity and to study the molecular mechanisms underlying its development.

Methods and Material: Male and female Wistar rats (300-400 g) were divided into 3 groups: 1) a control group treated with saline (ip. 6x1 mL/kg for 2 weeks), 2 a lower dose of PTX-treated (ip. 6x2 mg/kg for 2 weeks), and 3) a higher dose of PTX-treated group (ip. 6x4.5 mg/kg for 2 weeks). At week 19, cardiac morphology and function were assessed using echocardiography. A subset of the left ventricular samples isolated at week 20 were stained with picrosirius red and fast green to assess fibrosis, and UHPLC assays were performed to measure cardiac tryptophan metabolism and molecular markers associated with oxidative stress.

Results: The ejection fraction was significantly decreased only in the male PTX-treated groups compared to the control group, but a significant increase in E/e', indicative of diastolic dysfunction, and an increase in left ventricular collagen content were observed in all PTX-treated groups compared to the sex-matched control groups. Furthermore, a significant increase in 3-OH-kinurenine and oxidized glutathione was detected compared to the sex-matched control groups. In summary, both doses of PTX induced fibrosis and diastolic dysfunction in both sexes; however, only PTX males developed systolic dysfunction.

Conclusion: In addition, there was an increase in tryptophan catabolism and oxidative stress in the PTX-treated groups. Further molecular measurements are needed to understand the role of Trp metabolites in PTX-induced cardiotoxicity.

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Impact of age on the development of cardiovascular disorders following an external exposure to low doses of Caesium 137

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Background/Aims: Populations living near contaminated areas following nuclear accidents are chronically exposed to low doses (LD) of ionizing radiation (IR). However, the consequences at the cardiovascular level remain poorly evaluated. Epidemiological findings suggest a relationship between age and the onset of cardiovascular diseases (CVD) with higher cardiovascular morbidity and mortality rates in elderly subjects than in young subjects of the exposed populations. Our aim is to examine the effect of low to moderate doses of gamma rays on the development of late CVD, especially vascular remodeling, while highlighting the age-related health effects.

Methods and Material: 15 months-old male C57Bl/6J mice were exposed total body to gamma rays' doses ranging from 0.1 to 0.5Gy to mimic cumulated doses of IR chronic exposure. The analyses were carried out at 24-hours, 15-days and 3-months time points post-IR. The effects of radiation on the vascular system were assessed 1) by doppler ultrasound and Millar probe for functional parameters and 2) by immunolabelling and histological staining for morphology.

Results: 15 days post-exposure, a significant alteration of cardiac function was observed with an accelerated heart rate (472 BPM in non-irradiated mice (NIR) vs 517 BPM, p=0,04) and a decrease in left ventricular ejection fraction (69% vs 79% for NIR, p=0,026) at 100mGy. A significant enlargement of aortic diameter was also observed at 250mGy. Pulmonary artery systolic pressure increased significantly at 100 and 250mGy (24mmHg and 23mmHg respectively vs 17mmHg for NIR, P=0,02 and P=0,006), and this result was confirmed by analyzing pulmonary vascular remodeling. Immunolabelling of lung sections showed a significant increase in pulmonary arteries musculature at 100 and 250mGy (43% and 39% respectively vs 26% for NIR, p=0,004 and p=0,04).

Conclusion: These results show that early effects occur at doses as low as 100mGy, suggesting that exposure to low-intensity IR (<500mGy) induces structural and functional alterations that could participate in the vascular pathologies observed in elderly people living in contaminated areas. This study suggests that CVD bioindicators should be evaluated in low-dose exposed cohorts of all ages.

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Effect of inhibition of ultrarapid delayed rectifier potassium current (i_{kur}) by selective blockers on dog ventricle

Does I_{Kur} operates in ventricle?

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Background: The ultrarapid delayed rectifier potassium current (IKur), mediated by Kv1.5 channels, is traditionally thought to be exclusive to atrial tissues with minimal or no functional presence in ventricular myocytes. This notion shaped the development of XEN-D0103, a selective IKur inhibitor, as a potential therapy for atrial fibrillation with minimal proarrhythmic risk in ventricular tissues. However, emerging evidence challenges this belief, suggesting a potential role for IKur in ventricular muscle, reigniting debate over its functional presence and significance.

Objective: This study aims to confirm the expression of Kv1.5 channels in canine ventricular tissue, evaluate the electrophysiological effects of IKur inhibition on ventricular repolarization, and assess the specificity and broader implications of XEN-D0103 on cardiac ionic currents.

Methods: Immunocytochemistry: Kv1.5 channel expression was detected in canine ventricular myocytes. Electrophysiological Techniques: Conventional microelectrode methods measured action potential dynamics in right and left ventricular muscle.

Patch-clamp techniques evaluated the effects of XEN-D0103 on IKur and other key ionic currents (IKr, IKs, Ito, and IK1) in ventricular myocytes. Pharmacological agents including 4-aminopyridine (broad potassium channel blocker) and XEN-D0103 (IKur-specific blocker) were tested at varying concentrations.

Results: Immunocytochemistry confirmed abundant Kv1.5 expression in ventricular myocytes, challenging the prior belief of its atrial exclusivity. XEN-D0103 at concentrations below 1 µmol significantly prolonged APD90 in right ventricle (235±11ms to 254± 9 ms)left midmyocardial preparations(273±7 to 287±8 ms).4-Aminopyridine at concentration 100µM significantly prolonged APD90 in left midmyocardial preparations (273±12 to 288±14 ms).At higher concentrations (3–10 µmol), XEN-D0103 partially inhibited other potassium currents (Ito, IKr, IKs, and IK1).

At therapeutic levels, XEN-D0103 selectively inhibited IKur without affecting other major repolarizing currents, supporting its role as a targeted IKur inhibitor.

Conclusion: The findings underscore the presence of functional IKur in ventricular myocytes, demonstrating its influence on repolarization dynamics. While XEN-D0103 shows potential as a targeted therapy for atrial fibrillation, the prolongation of ventricular repolarization raises important questions about its antiarrhythmic and proarrhythmic implications. These results highlight the need for further investigation into IKur's therapeutic potential and safety in ventricular tissues.

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Blood-based microRNAs in post-stroke patients may modulate the antiplatelet potential in favour of outcome response – a follow-up analysis

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Background/Aims: MicroRNAs are small non-coding RNAs implicated mainly in post-transcriptional gene silencing by interacting with the untranslated region of the transcript. MiR-199a5p represents an anti-inflammatory player, serving versatile functions. We aimed to analyse blood-based microRNAs in post-stroke patients on dual or mono antiplatelet therapy (DAPT or aspirin (A) or clopidogrel (C) and their link to ischemic events during follow-up (FU).

Methods: A total of 60 patients were prospectively recruited. Composite vascular events (MI, TIA/stroke) were recorded during 18 months FU. Venous blood samples were taken from each participant, then miRNA expression was assessed by microarray analysis followed by real-time reverse transcription-polymerase chain reaction (RT-PCR) validation of particular miRNAs (miR1263p, miR2233p, miR243p, miR199a5p).

Results: Significant differences were observed in miR1263p (A<C<DAPT); miR199a5p (A<<DAPT), miR2233p (C<DAPT); and miR243p (A<C). Using DAPT, the expression of miR1263p ($p=0.017$) was significantly lower in patients with ichemic events during FU. In a subgroup of patients on both, statin and antiplatelet therapy, miR-199a-5p was repressed in those with ischemic events compared to uncomplicated cohort. In addition, miR-199a-5p was overexpressed in patients taking statin without ischemic events on FU ($P < 0.001$).

Conclusions: Our findings suggest that the abnormal repression of certain miRNAs may contribute to an increased risk of recurrent ischemic events. In contrast, the overexpression of miR1263p independently from statin therapy and miR-199a-5p in patients on statin may be involved in modulating the antiplatelet potential in favour of outcome response.

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Microglia modulate systemic inflammation-related central leukocyte recruitment and cerebral blood flow via interleukin-1

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Background/Aims: Microglia, the main immune cells of the brain, are closely associated with the vasculature and play a key role in modulating vascular responses. However, the mechanisms through which microglia modulate leukocyte recruitment into the brain and influence cerebral blood flow (CBF) during systemic inflammation remain poorly understood. To this end, we developed a sustained systemic inflammation model that leads to central leukocyte recruitment without any direct manipulation of the brain tissue.

Methods and Material: Mechanisms of central leukocyte recruitment were investigated by histology, flow cytometry and imaging after repeated intraperitoneal administration of LPS. Involvement of microglial IL-1α and IL-1β and vascular endothelial IL-1 receptor 1 (IL-1R1) was studied in conditional KO mice or after systemic blockade of IL-1R1. The role of microglia and border-associated macrophages (BAMs) were assessed in cell depletion experiments. To assess cerebrovascular reactivity, hypercapnia-induced vasodilatory response was measured *in vivo* by Laser Speckle Contrast Imaging (LSCI).

Results: Our findings revealed that sustained systemic inflammation altered microglial morphology and induced leukocyte recruitment to the brain. Paralelly, it increased overall microglial process-vascular interactions throughout the vasculature and microglial somatic associations particularly at the venous side. Downregulation of microglial P2Y12R by systemic inflammation was also observed and the regulatory functions of microglia were demonstrated on central leukocyte recruitment and cerebrovascular reactivity. Reduced hypercapnia-induced vasodilatory response under systemic inflammation involves microglia and endothelial IL-1R1 signalling. However, IL-1 that drives leukocyte recruitment seems to be only partially microglia-derived and partially IL-1R1 independent in our model. While microglia showed interactions with leukocytes crossing the vasculature, microglial depletion by PLX5622 reduced leukocyte recruitment, similarly as seen in microglial IL-1α or IL-1β KO mice. In contrast, depletion of BAMs by clodronate enhanced leukocyte recruitment suggesting a distinct role for BAMs than microglia during systemic inflammation. Importantly, depletion of microglia impaired hypercapnia-induced vasodilation, unlike depletion of BAMs.

Conclusion: Our findings indicate that microglia emerge as key modulators of central leukocyte recruitment and systemic inflammation-associated hypoperfusion, with distinct roles to BAMs.

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Microvascular remodeling of the cerebral venous system after cerebral ischemia

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Background/Aims: Understanding the mechanisms of cerebral microvascular angiogenesis is essential for cell therapy and neurovascular reconstruction after ischemic stroke. Using two-photon microscopy, we previously determined the spatiotemporal dynamics of cerebral capillary remodeling in a chronic hypoxia model in living mice. However, the induction of systemic hypoxia may differ from the pathophysiology of cerebral ischemia. Therefore, the aim of this study was to investigate cerebral microvascular remodeling after cerebral ischemia induced by a photothrombotic model as a more relevant animal model to promote cerebral ischemia-induced microvascular angiogenesis.

Methods: Male C57BL/6J mice (3 months old, N = 6) were used. A chronic cranial window was created over the left parietal region under isoflurane anesthesia using the Tomita-Seylaz method. After administration of the photosensitive synthetic dye Rose Bengal, green light was applied to a portion of the exposed cortical surface to induce focal cerebral ischemia. Before and after the induction of cerebral ischemia, images of the vascular structure on the cortical surface and in the parenchymal tissue were repeatedly obtained by stereomicroscopy and two-photon microscopy, respectively, up to 14 days after irradiation.

Results: Vascular structures on the cortical surface showed the absence of blood circulation around the irradiated area and progressive recovery from the border area by day 7. Vascular structures in the parenchyma showed that the images were significantly blurred due to leakage of contrast agent from the damaged vessels. However, by day 3 after irradiation, the leakage was normalized and vascular structures became visible. At this time, venous dilation and tortuosity were observed in the irradiated region. Between days 3 and 5, new vessels emerged from the parenchymal venules, and all the new vessels were found to sprout from the first or second branches of the parenchymal venules. It was consistently observed that cerebral angiogenesis occurred in the marginal and central veins within the ischemic regions, but not from the surrounding intact regions.

Conclusion: Focal cerebral ischemia induces microvascular remodeling and angiogenesis from the venous system, whereas the upstream arterial vessels were relatively preserved.

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Persistent constriction of cerebral veins caused by intermittent hypoxia

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Background/Aims: Intermittent hypoxia (IH) is a state of cyclic exposure to hypoxia and reoxygenation. Prolonged exposure to IH is a key feature of sleep apnea syndrome (SAS), which is known to cause cerebral hypoperfusion and ultimately cognitive decline. The cascade from IH to cognitive decline may involve IH-induced damage to vascular endothelial cells. However, the exact mechanisms of IH-induced changes in the cerebral microcirculation are not fully characterized. Therefore, we investigated the longitudinal effects of IH on cerebral microcirculation in experimental animals.

Methods: C57BL/6 male mice (11-17 weeks old, n = 5) that had undergone Tomita-Seylaz cranial window surgery on the left skull were used. The oxygen concentration in the animal cage was cyclically changed from 21% to 7%, and this IH exposure was repeated for 6 h during the day for 2 weeks. Before and after IH treatment, the vascular structure on the cortical surface and in the parenchymal tissue was imaged by stereomicroscopy and two-photon laser scanning fluorescence microscopy, respectively. Image analysis was performed to measure the diameter of blood vessels and to count the number of capillaries through the respective branches of parenchymal arterioles or venules. Local hemodynamics was evaluated by the arteriovenous transit time of a portion of fluorescent dye injected intravenously. In addition, vascular function was assessed by measuring the evoked response of cerebral blood flow (i.e., neurovascular coupling) in the somatosensory cortex using laser Doppler flowmetry during 20 seconds of mechanical stimulation of the contralateral whiskers.

Results: Chronic exposure to IH caused sustained constriction of brain surface veins, but not arteries. In the parenchyma, no changes in capillary number were observed, but a significant reduction in vessel diameter was observed on the venous side of the capillaries (6.9 µm and 5.5 µm before and after IH, respectively). Local blood perfusion, as measured by arteriovenous transit time, was significantly reduced after IH exposure. In addition, vascular function, as measured by neurovascular coupling, was consistently reduced by IH exposure.

Conclusion: Cerebral hypoperfusion induced by IH is associated with persistent cerebral venous constriction.

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The impact of vasodilation on perivascular spaces through in vivo two-photon microscopy

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Background: Brain clearance is crucial to brain homeostasis, and has gained much attention lately due to its possible involvement in the accumulation of proteins associated with neurodegenerative diseases. Perivascular spaces, together with the subarachnoid space, are considered a possible exit route for waste products from the brain into the lymphatic system and blood. In the present study, we focused on the possible connection between perivascular spaces of arteries and veins, and the impact of vasodilation on the perivascular space size.

Methods: A chronic cranial window was created above a branch of the middle cerebral artery in C57BL/6J mice. Following a recovery period of seven days, these mice were anesthetized with a mixture of ketamine, dexmedetomidine, and atropine. Mice were mechanically ventilated. 15 µl of Texas Red-dextran tracer was injected into the CSF via the cisterna magna, while 150 µl of FITC-dextran was injected retro-orbitally into the bloodstream. In vivo two-photon microscopy was then used to visualize the perivascular spaces around the blood vessels. Z-stacks were obtained after ventilation with a mixture of oxygen and air (50%/50%), as well as a after ventilation with a mixture of oxygen, air, and 5% CO_2 (47,5%/47,5%/5%) to induce vasodilation. Afterward, the perivascular spaces and blood vessels were manually segmented. All experimental studies were approved by the animal ethics committee of the University of Amsterdam, and were executed according to the European Union guidelines for the welfare of laboratory animals (Directive 2010/63/EU).

Results: Data revealed that perivascular spaces were more prevalent around arteries compared to veins in the subarachnoid space and around penetrating vessels. The perivascular spaces of arteries and veins were connected via the subarachnoid space. After the addition of CO_2, both arteries and veins dilated, though the effect was more pronounced in arteries. Vasodilation resulted in a decrease in perivascular space around arteries, while a no change was observed in perivascular spaces around veins.

Conclusion: Overall, these findings provide new insights into the anatomical structure of perivascular spaces and the effects of vasodilation on its size. Future research should focus on the effects of vasodilation on whole-brain solute exchange.

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Role of brain vascular senescence in age-related cerebromicrovascular dysfunction and vascular cognitive impairment

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Background/Aims: With advanced age, neurovascular dysfunctions manifest as neurovascular uncoupling, blood-brain barrier (BBB) disruption, and microvascular rarefaction. These changes substantially contribute to the pathogenesis of vascular cognitive impairment and dementias (VCID) and even Alzheimer's disease (AD). Our previous research established a link between vascular senescence-induced cerebromicrovascular dysfunction and cognitive decline in accelerated aging models. The objective of this study was to determine the timeline of age-related accumulation of senescent endothelial cells and their role in neurovascular dysfunction, microvascular rarefaction, BBB disruption, and cognitive decline.

Methods and Material: Transgenic p16-3MR mice were treated with senolytic therapy, allowing for the selective elimination of senescent cells. The p16-3MR model enables both the detection and depletion of senescent cells using Ganciclovir. Mice underwent multiple anatomical and functional assessments, including neurovascular coupling (NVC) measurements via laser speckle flowmetry, microvascular density evaluation and BBB permeability analysis using two-photon microscopy as well as spatial learning and memory performance assessment using the radial arm water maze (RAWM).

Results: Aging led to increased microvascular endothelial cell senescence, which was associated with impaired NVC responses, microvascular rarefaction, and BBB disruption. These changes correlated with cognitive decline in aged mice. Senolytic treatment in 18–19-month-old mice significantly reduced endothelial senescence, restored NVC responses, partially improved BBB integrity, and enhanced cognitive function.

Conclusion: Our findings demonstrate that targeting senescent endothelial cells can restore key neurovascular functions and improve cognitive outcomes in aging. Importantly, these results highlight the therapeutic potential of senolytic strategies in preventing or delaying cerebrovascular aging, which could have implications for early intervention in VCID.

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Traumatic Brain Injury induces senescence in the cells of the Neurovascular Unit

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Introduction: Traumatic brain injury (TBI) is one of the leading causes of disability. It induces vascular damage, accelerates cellular senescence, impairs repair mechanisms, contributes to inflammaging, and cognitive aging. Cellular senescence caused by double-stranded DNA damage was identified as the mechanism driven by brain dysfunction after TBI. This study aims to investigate the cellular senescence in neurovascular unit cells in response to traumatic brain injury in rat brain sections.

Methods and Materials: Marmarou weight drop model was used to induce TBI in rats. Animals were divided into 4 groups: severe TBI (STBI), mild TBI (MTBI), repetitive mild TBI (rMTBI), and control (rSHAM) at two time points: 24 hours and 4 weeks. Brain sections were co-stained with a senescence-specific marker (γH2AX) and cell type-specific markers (CD31, GFAP, Iba1, and PDGFRβ for endothelial cells, astrocytes, microglia, and pericytes) and imaged using epifluorescence and laser confocal microscopy.

Results: A statistically significant number of astrocytes were γH2AX positive in STBI after 24 hours of injury. Microglia were γH2AX positive in statistically significant numbers in STBI and MTBI after 24 hours. Senescent astrocytes and microglia disappeared after 4 weeks. No γH2AX positive pericytes were found in any of the experimental groups. Senescent endothelial cells appeared in response to TBI only after 4 weeks, but this was only statistically significant in the cortex in STBI. Behavioural testing found no significant changes.

Conclusions: Our data shows that TBI-induced senescence is cell type specific and its dynamics are opposite for glial cells and endothelial cells, while pericytes do not take part in this response at the time points tested.

All necessary ethical approvals have been received for the aforementioned research. This research has also been supported by the University Research Scholarship Program (EKÖP-2024) Grant (5K 102 A202).

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Chronic impairment of neurovascular coupling and cognitive decline in young survivors of severe traumatic brain injury

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Background: Severe traumatic brain injury (TBI) leads to chronic cognitive decline, imposing a significant societal burden. The regulation of cerebral blood flow (CBF) is critical for cognitive function, and acute disruptions in CBF regulation predict poor TBI outcomes. However, the long-term effects of TBI on CBF regulation and their association with cognitive function remain poorly understood.

Objective: This study aimed to investigate whether severe TBI results in chronic CBF dysregulation and whether this contributes to long-term cognitive deficits. Additionally, we examined the role of TBI-induced insulin-like growth factor 1 (IGF-1) deficiency in cerebrovascular dysfunction.

Methods: We assessed cognitive function, basal CBF (via phase contrast MRI), CBF autoregulation (via transcranial Doppler), and neurovascular coupling (NVC) in 33 TBI survivors (mean age: 37.6 years, ~10 years post-injury) and 21 age-matched healthy controls. Serum IGF-1 levels were also measured.

Results: TBI survivors exhibited significant impairments in memory and executive function compared to controls. While basal CBF and autoregulation remained intact, NVC responses were chronically impaired and correlated with cognitive deficits. However, IGF-1 levels did not differ between groups and were not associated with NVC impairment or cognitive function.

Conclusion: Our findings indicate that severe TBI results in chronic impairment of neurovascular coupling, which likely contributes to long-term cognitive deficits. These results highlight the need for further research to identify underlying neurovascular mechanisms and develop interventions to restore NVC and cognitive function in TBI survivors.

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The role of microglia in modulating neurovascular processes and functional connectivity

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Background/Aims: Microglia are the main immune cells of the brain, with emerging roles that extend beyond immune-related functions. Although they are known to shape neuronal function in both physiological and pathological conditions, their involvement in neurovascular processes has remained largely unexplored. Our recent findings demonstrate that microglia play a crucial role in modulating cerebral blood flow (CBF) and neurovascular coupling through purinergic signaling. The aims of this study were to investigate the mechanisms of microglia-vascular interactions and the role of microglia in modulation of functional connectivity during resting-state activity and sensory stimulation.

Methods: CBF changes were measured by Functional Ultrasound (fUS) and Laser Speckle Contrast Imaging through the intact skull bone, in real time. Functional connectivity patterns were investigated during resting state and whisker- or visual stimulation using fUS. The role of microglial actions was investigated after elimination of microglia by PLX5622 or by using P2Y12R KO mice.

Results: Strengthening our previous data in the barrel cortex, our present results show that microglia influence neurovascular coupling in the visual cortex during visual stimulation. Additionally, while resting-state functional connectivity remained unchanged in the absence of microglia or microglial P2Y12R, we observed impaired thalamocortical functional connectivity during somatosensory stimulation in both microglia-depleted and P2Y12R KO mice.

Conclusion: These findings suggest that microglia should be considered as an important modulatory cell type involved in physiological and pathological alterations of CBF. Our observations also emphasize the importance of microglia-mediated actions in maintaining normal functional connectivity within the thalamocortical network during somatosensory stimulation. Understanding microglial actions may facilitate the discovery of novel treatment opportunities in common neurological disorders.

This study was supported by „Momentum" research grant from the Hungarian Academy of Sciences (LP2022-5/2022 to A.D.), the European Research Council (ERC-CoG 724994 to A.D.), and the EKÖP-2024-108 (E. Cs.) New National Excellence Program of the Ministry for Culture and Innovation from the Source of the National Research, Development and Innovation Fund. All necessary ethical approvals for this research were obtained in accordance with institutional and national guidelines.

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Psalmotoxin-1 induced alteration of local field potentials and spiking activity during hypercapnia in the newborn pig cortex

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Background/Aims: Hypercapnia is a potent stimulus of the neurovascular unit resulting in increases in cerebral blood flow. Acid-sensitive ion channel 1A (ASIC1A) has been recently identified to play a critical role in the microvascular response to hypercapnia in adult mice, however, we found that the selective ASIC1A inhibitor psalmotoxin-1 (PcTx1) only partially affected this response in newborn pigs. As we noted a predominantly neuronal expression of ASIC1A in the piglet cerebral cortex, we hypothesized that the marked neuronal electrophysiological response to hypercapnia in the piglet may also be affected by the ASIC1A inhibitor PcTx1.

Methods: The experiments were performed on anesthetized, mechanically ventilated male piglets (n=7) in accordance with state regulations and ARRIVE guidelines. The animals were equipped with an open cranial window over the parietal cortex for multichannel electrode insertion. Graded hypercapnia was elicited 3 times with 5-10%CO_2 ventilation, first under control conditions, then during topical application of PcTx1 (10nM), and after toxin washout. The obtained local field potential (LFP) and neuronal firing data were analyzed using spike sorting methods and power spectral density (PSD) calculations (Klusta/MATLAB).

Results: First, graded hypercapnia caused a typical biphasic PSD response constituting an increase (5%CO_2) then a decrease (10%CO_2) in the δ and the θ range, always originating from the deep cortical layers gradually shifting upwards. PcTx1 application completely abolished the θ PSD elevation, however, the PSD depression was unaffected. Toxin removal resulted in long lasting depression of cortical neuronal activity. Population firing rates were increased to 10%CO_2, interestingly most conspicuously (3-times) in the presence of PcTx1. Again, toxin removal virtually abolished to hypercapnia especially in the upper cortical layers.

Conclusions: Our data highlight that pharmacological inhibition of ASIC1A with PcTx1 also affects the biphasic electrophysiological response of cortical neurons to hypercapnia. PcTx1 limited PSD increases to 5%CO_2 and caused increased spiking to 10%CO_2. Moreover, the toxin appears to induce long-lasting neuronal effects that persist after toxin removal. We conclude that the cortical neurons are likely active contributors to the normal neurovascular response to hypercapnia in the newborn pig, and the activation of ASIC1A is clearly involved in the response mechanism.

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Investigating the difference in retinal arterial and venular vasodilation in response to two different flickering light protocols

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Background: Flicker induced vasodilation (FIV) non-invasively assesses retinal blood vessel function. A flickering light results in vessels dilating due to an increase in retinal metabolic demand. There is no standardised protocol for this assessment. This research aims to compare the arterial and venular responses to two different flickering light protocols.

Methods: Participants underwent one FIV protocol in both eyes (Dynamic vessel analyser (DVA), Imedos). Protocol 1 used 50s baseline (non-flickering light), followed by three cycles of 20s flickering light, with 80s between each flicker. Protocol 2 consisted of 50s baseline, 40s flickering light and then 60s of non-flickering light. The protocols were randomly allocated to each eye. DVA software automatically calculated baseline diameter and % dilation compared to baseline diameter for a maximum of 5 artery and 5 vein segments. Only segments with a validity of >60% have been included and a mean of these valid segments was calculated.

Results: 58 participants had at least 1 valid artery segment for both protocols (median age (25^{th},75^{th} quartiles): 71(68,74)years, BMI: 28.3(24.5,31.7)m^2/kg, 69% with type 2 diabetes (T2DM)). There was no significant difference in arterial % dilation between the two protocols (protocol 1: 1.60(0.53, 2.57)% vs protocol 2: 1.23(0.18, 2.88)%, p=0.849 Wilcoxon signed rank test). 72 participants had at least 1 valid vein segment for both protocols (age: 71(69,74)years, BMI: 28.0(24.7,31.3)m^2/kg, 63% T2DM). Venular % dilation was significantly lower with protocol 1 compared to protocol 2 (protocol 1: 2.67(1.7, 3.62)% vs protocol 2: 3.50(1.93, 4.6)%), p=0.024).

Conclusion: Venular flicker induced vasodilation was different between the two protocols, with an increased dilation with the longer flicker period in protocol 2. There was no significant difference in artery vasodilation between the two protocols.

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Assessment of retinal microvascular blood flow by OCT angiography in patients with carotid stenosis

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Aims: Our aim was to analyze the effect of systemic risk factors on macular and peripapillary blood flow in the eyes of patients with significant carotid stenosis.

Methods: A total of 112 eyes of 56 patients with significant carotid stenosis were included in the study. Each subject underwent three imaging sessions, during which three consecutive OCTA images of the macular area and the optic disc were obtained. The first session was performed during the perioperative period, and the other two were performed during the first postoperative week and one month after surgery. Using the RTVue OCT angiography device with the AngioAnalytics software, we measured the capillary density of the peripapillary region as well as the superficial and deep layers of the macula, the central retinal thickness, and the size of the foveal avascular zone. The effects of systemic risk factors and carotid endarterectomy on retinal vascular density were analyzed using multivariable regression models, while also controlling for image quality.

Results: Among the systemic risk factors examined, decreased eGFR, hypertension, and carotid stenosis negatively affected retinal capillary density ($p<0.001$), while statin use was associated with significantly better capillary circulation after controlling for other parameters ($p<0.001$). Following carotid endarterectomy, a significant improvement in macular and peripapillary capillary density was observed in both the operated and contralateral eyes ($p<0.001$).

Conclusion: OCT angiography, as a fast, easily repeatable, and non-invasive examination, has proven to be suitable for monitoring microcirculatory changes in this patient group. Our findings clearly indicate that a systematic relationship exists between specific systemic risk factors and retinal blood flow in this patient population. Confirming previous results, image quality had a statistically significant impact on vessel density.

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Unraveling the role of Reduced Folate Carrier 1 (RFC1, SLC19A1) in retinal microvascular integrity: mechanisms and implications

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Background/Aims: The Reduced Folate Carrier 1(RFC1, SLC19A1/SLC19a1) is responsible for transporting folates via blood–brain barrier(BBB). The retina shares similarities with the brain, while the inner blood–retina barrier(BRB) parallels the BBB. Our previous studies demonstrated the presence of RFC1 in the inner BRB, revealing it as an acute regulator of barrier integrity in both healthy and ischemic retinas. *In vitro* studies suggested that RFC1 can transport 2'3'-cGAMP into cells and activate the cyclic GMP-AMP synthase(cGAS)-Stimulator of Interferon Genes(STING) pathway. Our aim is to investigate whether RFC1 affects inner BRB integrity through STING signaling pathways *in vivo* using mouse retinas.

Introduction: Studies suggest that excessive STING activation exacerbates CNS damage, but low-level activation supports vascular stability. Given RFC1's potential role in 2’3-cGAMP transport, its deficiency may reduce STING pathway activation.

Methods and Material: Experiments were performed according to Hacettepe University Animal Experimentations Local Ethics Board approval(No:2023/08-06). RFC1-targeted short interfering RNA (RFC1-siRNA) was delivered intravitreally for RFC1 knockdown(n=5), non-coding siRNA used as control. STING inhibitor C176(10 mg/kg in %2.5 DMSO) was delivered intraperitoneally for 3 consecutive days(n=6). STING agonist 2’3’-cGAMP(25 mM, 2 ul) was injected intravitreally(n=3). Analyses were performed using whole-mount retinas, sagittal eye sections, and isolated retinal microvessels. They were examined for endogen IgG, Claudin-5 or STING, IRF3, pIRF3, IFNβ proteins using immunofluorescence and WES(Western Electrophoresis System), the sensitive automated capillary-based Western blot technique.

Results: There were no significant differences between RFC1-siRNA and NTC groups for STING levels, IRF3/IRF3 phosphorylation ratio and IFNB levels. In C176-injected retinas, there was a trend toward a decrease in claudin-5 expression in microvessels, but IgG leakage was not prominent. An increase were shown in IRF3 phosphorylation ratio and IFNβ expression in intravitreally 2’3-cGAMP injected retinas and a significant reduction in STING expression, however, further studies are ongoing.

Conclusion: Our preliminary results suggest that RFC1 downregulation-induced barrier loss is independent of the STING pathway. The complex interplay between STING signaling and inner BRB integrity indicates that activation triggers inflammation, while inhibition subtly affects barrier proteins. RFC1's role in barrier function remains to be explored for insights into barrier-related diseases and potential therapeutic targets.

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Improved noise reduction in dynamic vessel analysis using heartbeat filtering and RPCA

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Background & Aims: Dynamic Vessel Analyzer (DVA) measures retinal vascular reactivity by tracking micrometric diameter changes in retinal vessels through fundus video recordings. However, the resulting multichannel time-series data is prone to noise, which is inadequately addressed by current preprocessing methods. Existing techniques lack standardization and may fail to capture the full dilation profile and vascular parameters. This study proposes a preprocessing pipeline designed to improve noise removal and enhance the accuracy of DVA-derived features.

Methods: We introduced a preprocessing algorithm that first applies heartbeat filtering to identify and remove oscillations at and above the individual heartbeat frequency, eliminating high-frequency noise while preserving the vascular response. Next, we performed Robust Principal Component Analysis (RPCA) separately on arterial and venous locus-based time series to extract the underlying dilation-constriction profile while further suppressing noise and artifacts. The proposed method was evaluated on DVA recordings acquired using the Imedos Protocol, comparing 11 dialysis patients and 34 healthy controls. The extracted vascular parameters included Baseline Diameter Fluctuation (BDF), Maximum Dilation %, Maximum Constriction %, Time to Maximum Dilation, and Dilation Amplitude % (DA%), which were compared between groups using ANCOVA with age as a covariate.

Results: RPCA-based preprocessing produced the clearest separation between patient groups. DA% extraction in arteries was most significant with RPCA ($p = 0.0240$), compared to polynomial regression ($p = 0.045$) and median smoothing ($p = 0.056$). Similarly, BDF in arteries was best distinguished using RPCA ($p = 0.0024$), while polynomial regression ($p = 0.0068$) and median smoothing ($p = 0.018$) showed weaker significance. In veins, RPCA was the only method yielding a significant BDF result ($p = 0.040$), whereas polynomial regression ($p = 0.30$) and median smoothing ($p = 0.20$) did not show significance.

Conclusion: Current DVA preprocessing lacks standardization, limiting the reliability of extracted vascular parameters. The novel RPCA-based approach showed clearer and more consistent distinctions between healthy and dialysis patients. These findings suggest that RPCA enhances preprocessing by improving the reproducibility and accuracy of DVA-based vascular assessments.

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The role of systemic microvascular function and large artery stiffness in cognitive function in older adults

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Background: The penetration of excessive pulsatile energy due to increased large artery stiffness is thought to damage cerebral small vessels and lead to reduced cerebral perfusion and ischaemia, potentially resulting in a cognitive decline. Although it is challenging to directly access brain microcirculation non-invasively in humans, skin microvasculature could offer an alternative site for systemic/generalised microvascular function. We determined in this study whether large artery stiffness and systemic microvascular function were associated with cognitive function in older adults, and also determined whether systemic microvascular function mediated an association between large artery stiffness and cognitive function.

Methods: Older adults with diverse cardiovascular risk (n=435, 68±9 yrs) participated in this study. Estimated pulse wave velocity (ePWV) as an indirect estimate of large artery stiffness was calculated from age and blood pressure. Systemic microvascular function was assessed by skin acetylcholine (ACh) and sodium nitroprusside (SNP) responses using iontophoresis, and expressed as the area under the curve of each vasodilator (total ACh and SNP responses, respectively). Validated cognitive tasks [Addenbrooke's Cognitive Examination Revised (ACE-R), Trail Making Test Part A (TMT-A) and Part B (TMT-B)] were administered to assess participants' cognitive function.

Results: One standard deviation (SD) increase in ePWV (indicating an increase in arterial stiffness) was associated with a lower ACE-R score [β=-0.124 (-0.241, -0.007), *p*=0.037], and a longer completion time in TMT-A [β=0.349 (0.236, 0.462), *p*<0.001] and TMT-B [β=0.344 (0.234, 0.454), *p*<0.001] after accounting for conventional cardiovascular risk factors. One SD increase in total ACh response (indicating better endothelial function) was also associated with a shorter completion time in TMT-B [β=-0.117 (-0.206, -0.029), *p*=0.010] after accounting for conventional cardiovascular risk factors. A mediation analysis revealed that the association between ePWV and TMT-B was partially mediated by total ACh response explaining about 3% of the total effect in the crude model which did not persist in the adjusted model.

Conclusion: Large artery stiffness and systemic microvascular function were associated with cognitive function in older adults independently of conventional cardiovascular risk factors. The result of mediation analysis suggests that large artery stiffness and systemic microvascular function may independently influence cognitive function in our cohort.

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Association between tissue metabolic rate of oxygen consumption in the skin and BMI: results from a Swedish cohort

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Background/Aims: The aim of this study was to analyze the association between tissue metabolic rate of oxygen consumption ($tMRO_2$) in skin, calculated from microcirculatory parameters, and BMI.

Introduction: Previous studies have shown that impaired microvascular function is associated with both cardiovascular diseases and cardiovascular risk factors such as obesity and diabetes. Oxygen extraction from the microvasculature is important for cell viability and metabolism is known to be abnormal in obesity and metabolic syndrome.

Methods and Material: Microcirculatory perfusion, oxygen saturation, and fraction of red blood cells were quantified using an optical system combining laser Doppler flowmetry and diffuse reflectance spectroscopy. Measurements were performed during baseline (5 min), occlusion (5 min), and post-occlusion phase (10 min) on the forearm of 3809 subjects (aged 50-65 years) as part of the Swedish CArdioPulmonary BioImage Study (SCAPIS). tMRO2 was calculated from the measured microcirculatory parameters using a two-compartment mathematical model and compared between different BMI groups (normal weight (nw): 18.5≤BMI<25, overweight (ow): 25≤BMI<30 and obese (ob) BMI≥30) with or without diabetes during baseline and post occlusion.

Results: There were significant differences in $tMRO_2$ both during baseline (mean values, nw: 0.54, ow: 0.57 ob: 0.59 µM/s) and post occlusion (nw: 0.46, ow: 0.49 ob: 0.53 µM/s) for the different BMI groups ($p<0.001$), where the highest tMRO2 values were found for the obese group. In a linear regression model, tMRO2 was associated with BMI for both baseline and post occlusion ($p<0.001$, $R^2 =0.008$ and $R^2=0.01$, respectively). There was also a significant difference between subjects with or without diabetes for the obese group (0.63 vs. 0.58µM/s (baseline) and 0.57 vs. 0.52µM/s (post occlusion), $p<0.05$). The difference was not significant for the other BMI groups.

Conclusion: Quantifying $tMRO_2$ in absolute units enables comparison across different individuals. Our findings show that a high BMI is associated with an increased skin metabolism in baseline, which could be attributed to an increased oxygen consumption by metabolically active cells in obesity and thus a dysfunctional high recruitment of capillaries already at rest. These results, along with further research, may establish $tMRO_2$ as a potential marker of microvascular dysfunction in cardiovascular risk assessment.

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Association between cardiovascular risk factors and microvascular flowmotion in a Swedish cohort study

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Background/Aims: Vasomotion-induced oscillations in vessel diameter give rise to fluctuations in microcirculatory blood flow. These fluctuations, called flowmotion, are affected by cardiovascular risk factors (CVRFs). We aim to further investigate the association between established CVRFs and flowmotion. To our knowledge this is one of the largest studies of flowmotion.

Methods: The study was a local add-on study in Linköping of the Swedish CArdioPulmonary bioImage Study (SCAPIS), a large cohort study of individuals aged 50-64 years. Microcirculatory measurements were performed on the volar forearm of 3809 individuals using a fiber optic probe integrating laser Doppler flowmetry and diffuse reflectance spectroscopy during a 5-minute baseline period, a 5-minute occlusion and 10 minutes post-occlusive period. Flowmotion analysis using wavelets was performed on data from baseline and from the post-occlusive phase. The post-occlusive phase was further divided into a recovery and a post-recovery steady-state phase. Relationships between risk factors and flowmotion were investigated using multivariate linear regressions.

Results: Data from 3169 individuals were included in the regression model. The CVRFs were age, BMI, diastolic and systolic blood pressure, sex, pre-diabetes, diabetes mellitus, and current smoking. The largest effect sizes were found for current smoking, diabetes mellitus, and male sex. Current smoking was associated with decreased flowmotion, with the largest effect sizes in the myogenic (-0.31 SD) and neurogenic (-0.28 SD) frequency interval during baseline, in the myogenic interval during recovery (-0.26 SD) and during post-recovery steady-state (-0.27 SD). In people with diabetes, flowmotion was decreased in the respiratory interval during recovery (-0.22 SD). For male sex, increased flowmotion was observed in the neurogenic and endothelial interval during baseline (+0.40 SD) and post-recovery steady-state (+0.42 SD).

Conclusion: The strongest associations between risk factors and flowmotion was observed for diabetes (decreased flowmotion), current smoking (decreased) and male sex (increased).

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Vasomotion in human arteries and their regulations based on ion channel regulations: over 10 years study

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Background: Vasomotion, the oscillation of vascular tone, facilitates the blood flow into organs. Generally, spontaneously contractile organs such as the heart, GI tract, and genitourinary tract produce rhythmic contractions that periodically exert or relieve pressure on their vessels to facilitate the exchange of various substances. Although first described over 150 years ago, the physiological and pathophysiological mechanisms of vasomotion remain poorly understood. This study aimed to elucidate underlying mechanisms and physiological function of vasomotion in human arteries. Methods: Conventional contractile force measurement, immunohistochemistry, and Western blotting were employed to study human left gastric artery (HLGA) and uterine arteries (HUA). RESULTS: Circular muscle of HLGA and/or HUA produced sustained tonic contraction by high K^+ (50mM) which was blocked by 2µM nifedipine. Stepwise stretch and high K produced nerve-independent spontaneous contraction (vasomotion) (around 45 % of tested tissues). Vasomotion was also produced by application of Bayk 8644, 5-HT, prostagrandins, oxytocin. It was blocked by nifedipine (2µM) and blockers of intracellular Ca^{2+} stores. Inhibitors of Ca^{2+}-activated Cl^- channels (DIDS and/or niflumic acid) and ATP-sensitive K^+ (K_{ATP}) channels inhibited vasomotion reversibly. Metabolic inhibition by sodium cyanide (NaCN) and several neuropeptides also regulated vasomotion in K_{ATP} channel-sensitive and -insensitive manner. Finally, we identified TMEM16A Ca^{2+}-activated Cl^- channels and subunits of K_{ATP} channels (Kir 6.1/6.2 and sulfonylurea receptor 2B (SUR2B)), and c-*Kit* positivity by Western blot. Vasomotion of HLGA was observed in both circular and longitudinal muscle of artery and it was regulated by stretch-activated channel, DIDS, cyclic nucleotides and so on. We conclude that vasomotion is sensitive to TMEM16A Ca^{2+}-activated Cl^- channels and metabolic changes in human gastric and uterine arteries. This phenomenon was observed in both circular and longitudinal muscle of artery. Vasomotion might play an important role in the regulation of microcirculation dynamics even in pacemaker-related autonomic contractile organs in humans.

Vasomotion; Human artery; metabolism; Cl^- channels

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Microvascular dysfunction is associated with incident cardiovascular disease: The Maastricht Study

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Background: Previous studies have shown that microvascular dysfunction may play an important role in the pathogenesis of cardiovascular disease. However, whether systemic microvascular dysfunction is associated with incident cardiovascular disease and whether it differs among coronary, cerebral, and peripheral artery disease remains unknown. We aimed to investigate the association of microvascular dysfunction with incident cardiovascular disease using multiple microvascular measurements in a population-based cohort study.

Methods: Among 4895 participants (mean age 59 years, 48.7% men, 21.4% with type 2 diabetes), we assessed microvascular dysfunction (MVD) at baseline using markers for cerebral small vessel disease, retinal diameters, flicker light-induced retinal microvascular dilation, plasma biomarkers, renal biomarkers, heat-induced skin hyperemia, skin microvascular flowmotion, and sublingual microvascular glycocalyx thickness, and density. We summarized these measures into a composite score of MVD. We used a self-reported questionnaire for the annual follow-up of incident cardiovascular disease. We performed Cox regression analyses and adjusted for age, sex, type 2 diabetes, waist circumference, systolic blood pressure, total-to-high-density-lipoprotein cholesterol ratio, smoking, alcohol intake, and the use of lipid-modifying and/or antihypertensive medication.

Results: Over a median follow-up of 9.11 [7.20, 10.17] years, 613 participants reported incident cardiovascular event. A higher MVD composite score was significantly associated with a higher risk of incident cardiovascular disease in the fully adjusted model (hazard ratio [HR] per SD=1.09, 95%CI [1.00, 1.18]). In addition, MVD was associated with peripheral arterial disease ([HR] per SD=1.13, 95% CI [1.00, 1.28]). While the associations of MVD with myocardial infarction (hazard ratio [HR] per SD=1.02, 95% CI [0.87, 1.18]) and cerebrovascular disease (hazard ratio [HR] per SD=1.09, 95% CI [0.95, 1.25]) were in the same direction, they did not reach statistical significance.

Conclusions: Microvascular dysfunction is associated with a higher risk of cardiovascular disease, independently of major cardiovascular risk factors. This was particularly apparent for peripheral artery

disease, but not for coronary heart disease and cerebral events. The present findings support the hypothesis that systemic microvascular dysfunction may contribute to the development of cardiovascular diseases.

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Capillary refill time changes across stages of inflammation in a human model of endotoxemia

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Background: The physiological responses to inflammatory activation vary between individuals. Blood pressure and heart rate are variably well compensated for by microcirculatory changes in severe inflammation, like sepsis. Thus, alterations in the microcirculation offer interesting complements to traditional vital signs, and the readily available cutaneous microcirculation has the potential to be used as a surrogate marker of systemic microvascular function in inflammation.

Here, we investigated microcirculatory changes in healthy volunteers with lipopolysaccharide (LPS) induced experimental endotoxemia to simulate sepsis. We focused on Capillary Refill (CR) time, which is often advocated for as an indicator of microcirculatory health in guidelines for sepsis care.

Objectives: The aim of this randomized, placebo-controlled, crossover study was to investigate how finger pulp CR times correlate to the inflammatory response induced by LPS injection in healthy volunteers.

Method: 15 (target: 25) healthy volunteers, 18-40 years-old (13 female, 12 male, mean age: 22, SD: 2 years) visited on two days. The participants were intravenously administered LPS (0.8ng/kg), or placebo (NaCl 0.9%), randomized between the two visits. CR time was measured at baseline (BL), and timepoint (T) 1.5 h, 3.5 h, 5 h after injection.

Results: In the placebo condition, median CR times fluctuated over 2 seconds (range: 2-4s). Upon LPS challenge, median CR times were; BL: 1.5 s (IQR 0.9-6.7), T1.5: 5.8 s (IQR 4.8-7) (significant increase compared to baseline (Wilcoxon matched-pairs signed rank test, $p<0.05$)), T3.5: 1.9 s (IQR 0.8-4.2), and T5: 1.1 s (IQR 0.6-1.3). Pairwise comparison of CR time in LPS and placebo showed significant difference at all corresponding time points except BL.

Conclusion: CR time has a wide inter- and intra-participant spread at baseline, challenging traditional assumptions about normal cut-off values. However, CR time does increase in a human model of experimental endotoxemia at peak immune response and shows interesting dynamics over the course of the inflammatory response.

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Porphyromonas gingivalis in cardiovascular tissues: implications of oral-systemic health

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Background: Cardiovascular disease (CVD) impacts approximately 500 million individuals worldwide and is responsible for nearly 20.5 million deaths each year. Research has linked periodontitis to an elevated risk of CVD, including conditions such as atherosclerosis, hypertension, endocarditis, and heart attacks. The severity of periodontitis appears to be directly associated with an increased likelihood of developing CVD.

Objective: This study aimed to assess the prevalence of *Porphyromonas gingivalis* (*P. gingivalis*) in cardiovascular (CV) tissue samples from adults, comparing individuals with a healthy periodontium, periodontitis, or edentulism. Additionally, the study investigated whether the presence of *P. gingivalis* varies by CV sample site and examined the likelihood of detecting *P. gingivalis* based on the patient's oral *P. gingivalis* status.

Methods: A systematic review adhering to PRISMA guidelines was conducted, and random-effects meta-analyses were used to estimate *P. gingivalis* prevalence in CV samples, with subgroup analyses included.

Results: A review of 75 studies found *P. gingivalis* in 13% of CV lesions. The prevalence was 11% (95% CI: 0.01-0.53, $I^2 = 0\%$) among individuals with healthy gums and 23% (0.13-0.36, $I^2 = 80\%$) in those with periodontitis. Due to limited data, statistical analysis for edentulous patients was not feasible.

Among individuals with periodontitis, *P. gingivalis* was most frequently detected in coronary arteries (36%, $I^2 = 77\%$). Other affected sites included the lower extremities (19%, $I^2 = 0\%$), carotid arteries (11%, $I^2 = 72\%$), and the aorta (5%, $I^2 = 82\%$). Insufficient data were available for mammary, cerebral, and heart tissue samples.

An odds ratio analysis from six studies revealed that the likelihood of identifying *P. gingivalis* in CV samples was eight times higher in individuals with oral *P. gingivalis* presence.

Conclusion: The highest prevalence of *P. gingivalis* was observed in coronary arteries. These findings underscore the potential role of oral health in preventing or slowing CVD progression, highlighting the importance of preoperative dental care in CVD patients.

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Metformin diminishes the serotonin-induced vasoconstriction in foetal vessels and modifies the gene expression in foetal endothelial cells

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Background/Aims: Gestational diabetes mellitus (GDM) is the most common metabolic complication in pregnancy and a major risk factor for endothelial dysfunction, hypertension, and cardiovascular diseases in both mother and offspring in later life. In Germany, treatment options are limited to dietary management and insulin therapy. Metformin has not yet been approved as monotherapy for GDM due to limited understanding of its effects on the foetus. Therefore, we aim to investigate the influence of metformin on foetal endothelial function and its potential to mitigate endothelial dysfunction associated with GDM.

Introduction: Metformin is a well-known first-line treatment for type 2 diabetes mellitus (T2DM). It has been demonstrated to improve insulin sensitivity and endothelial function in T2DM. We aimed to determine whether metformin exerts similar effects on foetal endothelium in GDM.

Methods and Material: Foetal vessels were isolated from placentas of normoglycaemic (NG) (n=4), insulin-treated GDM (iGDM) (n=5), and diet-treated GDM (dGDM) (n=3) mothers. Clinical data was collected from patients. The vessels were incubated for 24 hours with/without metformin, and endothelial function was assessed using a Mulvany myograph. Contraction was induced with serotonin, followed by vasorelaxation with increasing concentrations of insulin. In addition, human umbilical vein endothelial cells (HUVECs) were isolated and incubated with metformin for 24 hours before RNA and protein extraction. Normoglycaemic HUVECs were treated with metformin and/or activated with VEGF. Gene expression was quantified by qPCR.

Results: Patients with iGDM had a higher BMI than NG and dGDM. iGDM vessels without metformin showed reduced vasorelaxation with insulin compared to NG, suggesting an early sign of endothelial dysfunction in iGDM. Metformin-incubated vessels exhibited a reduced response to serotonin, with a smaller maximum contraction. The maximum insulin-induced relaxation was diminished in metformin-treated iGDM and NG groups. In addition, VEGF-treated HUVECs showed increased KLF2 and PGC1[illegible] expression compared to controls, whereas VEGF + Metformin treatment did not alter KLF2 expression relative to controls.

Conclusion: The present findings suggest metformin influences serotonin-induced endothelial responses in both NG and GDM vessels. Ongoing studies investigate the serotonin uptake in endothelial cells in the presence of metformin and gene expression in GDM-impaired HUVECs treated with metformin.

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Differences in skin blood flow response to graded cycling in type 1 diabetic individuals compared to healthy controls

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Background and Aims The characteristic microvascular complications of type 1 diabetes (T1D), such as retinopathy or nephropathy, are the result of impaired endothelial function. Exercise-induced vasodilation in the skin microcirculation is attributed to endothelial control and could serve as an early marker for impaired microvascular control. Therefore, the aim of our study was to investigate the effect of exercise on skin blood flow in T1D individuals compared to healthy peers.

Methods Nine recreational athletes with well-controlled T1D and nine age-, gender- and maximal aerobic performance-matched healthy controls performed short-term submaximal graded cycling (GC). Arterial blood pressure and laser Doppler skin blood flow in the fingertip (LDFp) and forearm (LDFf) were measured at the end of 20 minutes of passive recovery after cycling. Mean arterial pressure (MAP) and cutaneous vascular conductance values for both skin sites (CVCp, CVCf) were calculated. The measured parameters were ln-transformed for normalisation.

Results In T1D, GC resulted in significantly lower lnLDFf (2.3±0.4 in T1D; 3.5±1.1 in healthy; p=0.006) and lnCVCf during recovery (-2.4±0.4 in T1D; -1.1±1.2 in healthy; p=0.009) compared to the healthy group. There were no differences between the groups for lnLDFp, lnCVCp and MAP.

Conclusions In T1D, cutaneous perfusion of the forearm, but not the fingers, is attenuated in response to exercise. This impaired vasodilation response suggests early endothelial dysfunction in the T1D group. The ability to dissipate heat through increased blood flow through arterio-venous anastomoses, which is present in the finger pulp, is preserved in T1D. Further studies are needed to uncover the mechanisms underlying our results.

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The role of endothelin-1 in fetal endothelial dysfunction in gestational diabetes mellitus

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Background/Aims: Gestational diabetes mellitus (GDM) is one of the most common pregnancy complications and a major risk factor for endothelial dysfunction, hypertension and cardiovascular diseases in later life. Endothelin-1 (ET-1), a potent vasoconstrictor, plays an essential role in vascular homeostasis. Moreover, ET-1 induces the endothelial LOX-1 (lectin- like oxidized low-density lipoprotein receptor 1). We hypothesise that ET-1 mediates endothelial dysfunction and contributes to vascular damage and impaired vasoconstriction in GDM.

Methods: We obtained maternal and fetal vessels from human placentas of normo-glycaemic mothers (n=25) and patients with insulin-treated GDM (iGDM) (n=10) or diet-treated GDM (dGDM) (n=5). Groups were defined based on oral glucose tolerance test and clinical data of mothers and newborns. The vascular function of fetal vessels from mothers with iGDM, dGDM or normoglycaemic controls was analysed using the Mulvany Myograph. Gene expression was quantified by real-time PCR from fetal vessels of the cotyledon base and maternal spiral arteries of the patients.

Results: A significant overexpression of LOX-1 was observed in the fetal placental vessels of iGDM patients compared to normoglycaemic mothers. When comparing soluble LOX-1 protein levels in peripartum serum samples, an increase was noted in the iGDM group compared to the control group ($p = 0.1$). Endothelin-mediated vasoconstriction was present in fetal arterial placental vessels from the chorionic base across all groups. The ET-1 mediated vasoconstriction was reduced in patients with iGDM and dGDM compared to normoglycaemic controls. No significant differences in mRNA expression of endothelin receptor A (EDNRA) and endothelin receptor B (EDNRB) were found between GDM patients and controls. However, EDNRB expression was higher than EDNRA expression in fetal placental vessels across all groups. This may indicate a fetal compensatory mechanism in response to impaired vascular function in fetal placental arteries.

Conclusions: We could show that GDM impairs the vascular ET-1 system leading to altered endothelial function of fetal placental arteries. The LOX-1 receptor seems to play a key role in endothelial dysfunction in GDM. Therefore, the interplay between LOX-1 and ET-1 could be a promising target in the development of therapeutic strategies for GDM.

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Photobiomodulation therapy for diabetic erectile dysfunction: enhancing microvascular function and neurovascular regeneration

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Background/Aims: Diabetes-induced erectile dysfunction (ED) is often resistant to phosphodiesterase type 5 inhibitors due to significant vascular and neuronal impairments. Light-emitting diode (LED) therapy, emitting light at specific wavelengths, can stimulate mitochondrial metabolism and promote tissue regeneration. Here in this study evaluate the efficacy of light-emitting diode (LED) therapy in mitigating diabetes-associated ED by focusing on mechanisms related to cellular and tissue repair.

Methods: Diabetes mellitus (DM) was induced in 8-week-old C57BL/6 mice via streptozotocin (STZ) injection. Diabetic mice were divided into five groups: control, DM + heat treatment, DM + RED LED (660 nm), DM + near-infrared (NIR) LED (830 nm), and DM + combined RED and NIR LED. Mice underwent LED therapy daily for 30 minutes over 10 days across two weeks. Erectile function was assessed through intracavernous pressure (ICP) measurements, and molecular and histopathological analyses were performed to investigate neurovascular repair mechanisms.

Results: Diabetic mice showed significantly reduced ICP, which LED therapy improved, with red and NIR treatment restoring 90% of normal levels. Histology revealed increased endothelial cells, pericytes, angiogenesis, and neural regeneration. Molecular analysis showed upregulated neurotrophic factors (NGF, NT-3, BDNF), angiogenic markers (VEGF, eNOS), and phosphorylated PI3K, alongside reduced apoptosis and enhanced proliferation in cavernous tissue. LED therapy modulated neuroinflammation, promoting neural and vascular repair, with combined red and NIR wavelengths demonstrating superior efficacy through synergistic effects.

Conclusion: Low-level combined red and NIR LED therapy represents a promising, non-invasive treatment for diabetes-associated ED, addressing its underlying vascular and neuronal impairments. This novel approach may provide an alternative for cases resistant to conventional PDE5 inhibitors.

Key Words: Photobiology, Neurogenesis, Angiogenesis, Erectile dysfunction, Diabetes

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Circumferential radiofrequency electric field therapy improves diabetic lower extremity microcirculatory disorders

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Objective: To evaluate the efficacy and safety of circumferential radiofrequency electric field therapy for patients with diabetic lower extremity microcirculatory disorders ($TcPO_2$ 10-40 mmHg), and to provide evidence for non-invasive treatments in the prevention and management of diabetic foot.

Methods: A multicenter, randomized, placebo-controlled design was used. Currently, 24 patients have completed treatment (13 in the intervention group, 11 in the control group). The intervention group received 200 W continuous mode circumferential radiofrequency electric field therapy, while the control group used a sham device (no electric field output when powered on). The primary outcome measure was the change in transcutaneous oxygen pressure ($TcPO_2$) in the lower extremities before and after treatment. Secondary outcome measures included the Toronto Clinical Scoring System (TCSS) and other relevant indicators.

Results: Preliminary analysis showed that after 8 weeks of intervention, $TcPO_2$ in the intervention group significantly increased compared to baseline (32.5±5.1 mmHg → 44.7±4.3 mmHg), with a statistically significant increase ($P<0.01$). No statistically significant increase was observed in the control group ($P>0.1$). After 8 weeks of intervention, the difference in $TcPO_2$ values between the control and intervention groups was statistically significant ($P<0.01$). The TCSS score showed improvement compared to baseline, with a statistically significant decrease in the intervention group ($P<0.05$). No significant change was observed in the control group ($P>0.1$). Additionally, symptoms related to lower extremity microcirculatory disorders improved significantly in the intervention group compared to the control group, with no treatment-related skin burns or other adverse events reported.

Conclusion: Circumferential radiofrequency electric field therapy can safely and effectively improve the oxygenation status of diabetic lower extremity microcirculation, with significant early efficacy. Due to the limited number of cases currently enrolled, the long-term stability of outcome measures such as $TcPO_2$ needs further confirmation. Further large-scale studies are necessary, but this study, using a concealed randomization and single-blind design to control bias, provides a methodological foundation for future large-sample validation with a standardized protocol (200 W/25 minutes/day).

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Endothelial glycocalyx degradation in a mouse model of renal ischemia-reperfusion injury: potential protection with recombinant syndecan-1

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Background & Aims: The endothelial glycocalyx (EG) is a crucial functional and protective layer lining the inner face of blood vessel, composed of proteoglycans like syndecan-1, glycosaminoglycans, and glycoproteins. Damage to this structure during inflammation, or as seen in most cardiovascular diseases such as kidney ischemia-reperfusion (I/R) injury, largely contributes to endothelial dysfunction and comes with the release of glycocalyx components into circulation. This study aimed to explore the potential protective effects of recombinant syndecan-1 in preserving EG integrity during renal I/R injury to slow vascular complications, inflammation and damage to the tissue.

Methods: Using a mouse model of renal I/R injury with contralateral nephrectomy, we assessed EG degradation 24 hours post event, and the incorporation of a recombinant syndecan-1 tagged with 6X histidine (kindly provided by Dr Michael Goligorsky, New York Medical College). Methods included immunofluorescence for EG components labeling (wheat germ agglutinin, hyaluronan binding protein, heparan sulfate antibody) and confocal microscopy for analysis, western blotting to detect the 6X histidine-tag, ELISA to measure biomarkers of EG shedding (syndecan-1, hyaluronan, and heparan sulfate) and atomic force microscopy (AFM) to evaluate glycocalyx size (aorta).

Results: While 6X histidine-tag was detected using western blotting analysis, direct incorporation of recombinant syndecan-1 into the endothelium could not be confirmed. Immunolabelling and confocal microscopy revealed glycocalyx degradation in kidney vessels post-I/R, with significant shedding of hyaluronan into the bloodstream; heparan sulfate showed the same tendency while non significantly different. ELISA confirmed a substantial increase in hyaluronan and syndecan-1 shedding after I/R. At systemic level, i.e. in the aorta, renal I/R event significantly decreases the size of the EG, and this appears significantly mitigated after supplementation with recombinant syndecan-1 (AFM).

Conclusion: Our findings highlight EG degradation following renal I/R injury, particularly through hyaluronan shedding. However, even if an interesting trend is observed, no significant protective effect of recombinant syndecan-1 was observed. Nevertheless, in aorta, the repercussion of renal I/R event highlights significant degradation of the EG and a significant protective effect of recombinant syndecan-1. Further research is needed to elucidate its potential therapeutic role in preserving EG integrity and reduce endothelial dysfunction.

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The endothelial glycocalyx as mechanosensor of shear stress: functional and morphological investigation in mouse arteries

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Background/Aims: The endothelium plays key roles in vascular physiology, including the regulation of vascular tone. Thanks to its proximity with blood, endothelial surface is able to sense hemodynamic forces and initiate the endothelium-dependent relaxing pathways (NO, prostacyclin and EDH pathways). The endothelial glycocalyx (EG) is currently considered as a major mechanosensor of shear stress. Its involvement in nitric oxide (NO) pathway initiation was previously described but the relation between EG and endothelial dependent-hyperpolarization (EDH) pathway remains unclear. In this study, both pathways are investigated under flow conditions in 2 types of vessels: carotid (conductive) and mesenteric (resistance) arteries.

Methods and Materials: Enzymatic degradation of the EG was induced in mice carotid and mesenteric arteries using heparinase III from *Flavobacterium heparinum* (2U), hyaluronidases from bovine testes (1000U) or *Streptomyces hyalurolyticus* (5U), perfused into isolated vessel. Degradation of the EG was confirmed thanks to immunofluorescence staining and confocal microscopy analysis. Then, using pressure myography, the flow-dependent endothelial response of treated mice carotid and mesenteric arteries was studied. To evaluate endothelial health following enzymes perfusion, permeability and acetylcholine-induced vasodilation were assessed.

Results: The immunostaining of the EG confirmed its enzymatic cleavage (≃50% in both vessels). Flow-induced vasodilation after EG degradation, was strongly reduced (≃80%) in mesenteric arteries. Interestingly, the EDH pathway was particularly affected unlike the NO pathway. On the other hand, the response to flow of carotid arteries was maintained but the NO-dependent vasodilation was significantly decreased (≃40%). Regarding the EDH pathway, no difference was observed between treated and untreated vessels. For both vessels, the blood barrier permeability and the agonist-dependent dilation were not impacted.

Conclusion: This study confirms the essential role of the EG in mechanotransduction and establishes the interplay between EDH pathway and EG-dependent sensing of shear stress. Furthermore, major differences between conductive and resistance arteries were demonstrated. These findings reinforce the need to preserve EG or to restore it in patients suffering from cardiovascular diseases.

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Brain death-induced renal injury in pigs: endothelial dysfunction, glycocalyx alteration and the potential protective role of Tacrolimus

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Background & Aims: Brain death (BD) triggers a cascade of systemic disturbances that severely compromise kidney function and structural integrity, presenting challenges to transplantation success. This study aimed to evaluate the protective effects of tacrolimus (FK506), an immunosuppressive drug, in attenuating BD-induced renal injury. Using a 4-month-old female porcine model, we investigated the impact of FK506 pretreatment on rena and endothelial alterations associated with BD.

Methods: Piglets were divided into three groups: BD placebo-pretreated (n=9), BD tacrolimus-pretreated (n=8; 0.25 mg/kg the day before surgery, 0.05 mg/kg one hour before BD induction), and control (n=7, no BD induction). We assessed renal function, inflammation, apoptosis, leukocyte infiltration, and endothelial glycocalyx integrity to determine FK506's protective effects.

Results: BD led to significant renal deterioration, including increased proteinuria, induction of apoptosis in vessels, glomeruli, and tubules, leukocyte infiltration, and endothelial glycocalyx degradation in kidney vessels. Importantly, treatment with FK506 demonstrated significant efficacy in attenuating these adverse effects. FK506 helped reduce apoptosis, maintain glycocalyx integrity, regulate neutrophil infiltration, and mitigate renal injury following BD.

Conclusion: These findings highlight the potential of FK506 pretreatment as a novel strategy to improve organ preservation. By maintaining endothelial function and reducing inflammation, FK506 may enhance graft viability and long-term transplant outcomes, offering a promising approach to optimizing kidney transplantation.

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Unraveling the relationship between the ordered water layer and blood-brain barrier function via lab-on-a-chip technology

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Background/Aims: The endothelial glycocalyx (EGL) is a carbohydrate-rich layer located on the luminal surface of endothelial cells and plays a critical role in maintaining the integrity of biological barriers, including the blood-brain barrier (BBB). The structured water layer within the EGL is thought to be crucial for barrier function. This study investigates the effects of a chaotropic agent, such as urea, on the integrity of the BBB by disrupting the ordered water within the glycocalyx.

Methods: A lab-on-a-chip device integrated with electrodes was developed to monitor the integrity of the BBB in real-time. Human brain endothelial cells were co-cultured with bovine pericytes to establish a dual co-culture BBB model. Electrical impedance spectroscopy and permeability assays were used to assess barrier integrity under dynamic flow conditions. Urea, a chaotropic agent known to disrupt hydrogen bonding in water, was tested to evaluate its impact on BBB function.

Results: Urea treatment significantly reduced the measured impedance values, suggesting a weakening of the endothelial barrier. Further permeability assays revealed an increased paracellular transport of fluorescent dextrans (FD4) and transcellular transport using Evans Blue Albumin (EBA), supporting the hypothesis that chaotropic agents disrupt the barrier integrity.

Conclusion: Our study underscores the pivotal role of the structured water layer within the endothelial glycocalyx in maintaining the integrity of the blood-brain barrier. Disruption of this water layer, as seen with chaotropic agents like urea, compromises barrier function and may have significant implications for drug delivery across the BBB. These findings highlight the importance of the biophysical properties of the EGL and its water structure in regulating endothelial barrier function, offering new insights into potential strategies for enhancing drug delivery to the brain.

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Some hemorheological factors in experimental hemorrhagic shook

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Background/Aims: the problem of studying hemorrhagic shock in modern biomedicine is especially relevant. Treatment of patients with massive blood loss leading to the development of hemorrhagic shock requires special attention. Improvement of early diagnostics and correct treatment helps to reduce mortality and disability. The main reason for the high level of mortality is disorders of the adaptation mechanisms of the cardiovascular system, leading to tissue hypoperfusion, impaired oxygen transport and the development of tissue hypoxia. It is clear that blood hemorheology is impaired in this case. The main goal of therapy for posthemorrhagic conditions is, first of all, to restore the functional activity of the cardiovascular system and ensure an adequate level of oxygen delivery to tissues, i.e. normalization of hemorheological properties of blood. The purpose of our experiments was to study the hemorheological properties in animals with hemorrhagic shock.

Methods and Material: The experiments were performed on 18 outbred rats: Group I - control group (8 animals); Group II — animals with hemorrhagic shock, which were oxygenated after 15 minutes of shock. In animals with hemorrhagic shock, the erythrocyte aggregation index and erythrocyte deformation index were determined as the main parameters of hemorheology before and after oxygenation.

Results: In animals with hemorrhagic shock, compared to the control, erythrocyte aggregation sharply increased and erythrocyte membrane deformability decreased. After oxygenation (5th min), a sharp tendency to normalize erythrocyte aggregation was noted.

This is the basis for improving hemodynamic parameters, which contributes to the restoration of the functional activity of the myocardium and a decrease in the manifestations of tissue hypoxia.

Conclusion: In experimental hemorrhagic shock, blood hemorheology is disrupted due to increased erythrocyte aggregation. Oxygenation leads to an improvement in the hemorheological properties of the blood, which has a positive effect on the overall hemodynamics.

Acknowledgment: This work was supported by Shota Rustaveli National Science Foundation of Georgia (FR-24-389).

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Modulation of mitochondrial consequences in organ transplantation by methane enrichment of preservation solution

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Background/Aims: During prolonged organ preservation, cold ischemia leads to ATP depletion, mitochondrial dysfunction, and membrane damage, resulting in post-transplant organ dysfunction. In previous experiments, we demonstrated the pleiotropic organ-, cell-, and mitochondria-protective effects of methane gas (CH_4). In this study, we examined the effects of CH_4 on mitochondrial respiration in kidneys during clinically relevant static cold storage.

Methods and Material: Bilateral nephrectomy was performed on anesthetized ventilated pigs (n=4). The kidneys were immediately perfused and then stored in preservation solution at 4°C. The right kidneys were stored in histidine-tryptophan-ketoglutarate (HTK/Custodiol) solution, while the left kidneys were stored in HTK solution enriched with methane gas (2.2% CH_4-air normoxic mixture at 1.5 l/min flow for 15 minutes in 1 liter of Custodiol). After 24 hours of preservation, mitochondrial basal respiration, complex-I- and II-dependent oxidative phosphorylation (CI- and CII-linked OXPHOS), and cytochrome c oxidase (Cyt-C – complex IV; CIV) activity were measured in cortical and medullary homogenates using high-resolution respirometry (Oroboros O2k, Innsbruck, Austria). The integrity of the outer mitochondrial membrane was tested by addition of exogenous Cyt-C.

Results: Compared to cold HTK/Custodiol storage, preservation in CH_4-enriched HTK solution significantly improved CI- and CII-linked OXPHOS in both kidney cortex and medulla, and enhanced CIV activity in medullary samples. The presence of CH_4 also ameliorated the increase in the outer mitochondrial membrane permeability in both regions (cortex: 127±10% vs 58±23%, medulla: 140±25% vs 69±20%; $P<0.005$).

Conclusion: Enriching the preservation solution with CH_4 improves mitochondrial dysfunction and reduce membrane damage caused by cold ischemia. It is hypothesized that the reduction in Cyt-C release might limit the activation of apoptotic pathways, and thus the preservation of OXPHOS function may contribute to the attenuation of organ damage during kidney transplantation.

Fundings: 2024-1.2.3-HU-RIZONT-2024-00033, NKFI-149858, EFOP-3.6.2-16-2017-00006 and SZTE SZAOK-KKASZGYA2023/5S775

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Kynurenic acid-based therapies in sepsis: A focus on microcirculation and bioenergetics

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Background/Aims: Sepsis, a severe and life-threatening condition characterized by a dysregulated immune response to infection, often results in multi-organ failure. Microcirculatory and mitochondrial dysfunctions are key contributors to the progression of sepsis, leading to poor oxygen delivery and utilization. This study aimed to investigate the effects of kynurenic acid (KYNA), an N-methyl-D-aspartate receptor antagonist, and its synthetic analog SZR-72 on microvascular and mitochondrial impairments in a clinically relevant rodent model of intraabdominal sepsis.

Methods and Material: Male Sprague–Dawley rats were subjected to fecal peritonitis to induce sepsis (n=8) and treated with KYNA or SZR-72 (n=8 per group, 160 µmol kg^{-1} each, 16 and 22 h after induction). Mitochondrial oxygen consumption and mitochondrial membrane potential (ΔΨmt) were evaluated by High-Resolution FluoRespirometry in liver homogenates while the ratio of perfused vessels (PPV) of the ileal serosa was determined with the intravital imaging technique. Plasma endothelin-1 (ET-1), IL-6, intestinal nitrotyrosine (NT), and xanthine oxidoreductase (XOR) enzyme activities were assessed as inflammatory markers.

Results: We found that KYNA significantly improved microcirculatory function, as shown by an increase in the proportion of perfused vessels (PPV) and a reduction in perfusion heterogeneity. However, KYNA showed mild protective effects on mitochondrial function. In contrast, SZR-72 preserved mitochondrial bioenergetics by improving complex I- and complex II-linked oxidative phosphorylation (OXPHOS) capacities and restoring mitochondrial membrane potential. Both treatments effectively reduced systemic inflammation, as indicated by decreased levels of endothelin-1 (ET-1), interleukin-6 (IL-6), and nitrotyrosine.

Conclusion: These findings highlight the distinct therapeutic potentials of KYNA and SZR-72 in sepsis treatment. KYNA primarily targets microcirculatory dysfunction, while SZR-72 acts on mitochondrial respiration. The complementary actions of these compounds suggest that combined therapies could offer a promising approach to mitigating sepsis-induced organ failure by addressing both microvascular and mitochondrial dysfunctions.

Fundings: EFOP-3.6.2-16-2017-00006 and SZTESZAOK-KKASZGYA2023/5S775

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Possible epigenetic effects of vitamin C in the pathomechanism of Arterial Tortuosity Syndrome

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Background/Aims: Arterial Tortuosity Syndrome (ATS) is a rare, recessively inherited connective tissue disorder characterized by the tortuosity of large arteries and aneurysm formation. ATS is caused by a loss-of-function mutation in the gene encoding GLUT10 (*SLC2A10*), which transports the oxidized form of vitamin C, dehydroascorbate, across endomembranes. Recent studies suggest that ascorbate acts as a cofactor for DNA and histone demethylases (e.g. TET enzymes), playing a role in epigenetic regulation. Altered epigenetic patterns have been observed in ATS patients. We hypothesized that altered nuclear accumulation of ascorbate may occur in ATS, disrupting DNA and histone demethylation, leading to altered epigenetic patterns that may contribute to the pathomechanism of ATS. Our aim was to establish a mouse model of ATS by generating GLUT10-deficient mice, that are also unable to synthesize vitamin C (DKO); and to study the subcellular ascorbate levels and changes in the expression of vitamin C-dependent epigenetic enzymes.

Methods: Mice were divided into two dietary groups, one receiving vitamin C supplementation and one without dietary vitamin C intake. Vitamin C levels in mouse brain, liver and liver subcellular fractions were measured by HPLC-UV/DAD. The expression of TET enzymes was examined by qPCR. Protein expression of TET1 was also studied by Western blot analysis. Global DNA methylation and hydroxymethylation were examined by dot blot analysis.

Results: Ascorbate levels were decreased in DKO mice without dietary vitamin C intake, but could be restored by vitamin C supplementation. Analysis of subcellular fractions revealed that the nuclear ascorbate concentration was lower in DKO mice, even with vitamin C supplementation. The mRNA and protein expression of TET1 was significantly higher in the brain of DKO mice without vitamin C supplementation compared to wild type mice. There was no significant difference in the global DNA methylation, however, we detected lower levels of DNA hydroxymethylation in the brain of DKO mice.

Conclusion: In conclusion, DKO mice exhibited altered ascorbate distribution and vitamin C-dependent enzyme expression, which might initiate the novel idea of considering ATS as an ascorbate compartmentalization disorder.

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Bone morphogenetic protein 2 rescues neurogenic abnormalities and angiogenic factors in mice with bilateral cavernous nerve injury

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Background/Aims: Bone morphogenetic protein 2 (BMP2), a key isoform within the bone morphogenetic protein family, plays a critical role in promoting angiogenesis and peripheral nerve regeneration. However, the specific role of BMP2 in neurogenic erectile dysfunction (ED) remains unclear. This study aimed to explore the therapeutic efficacy of exogenous recombinant BMP2 protein administration in restoring erectile function in a mouse model of cavernous nerve injury (CNI)-induced ED.

Methods: Eight-week-old male C57BL/6 mice were used to evaluate BMP2 expression and erectile function following CNI. Western blotting and immunofluorescence staining were employed to assess BMP2 levels in corpus cavernosum tissues from both sham-operated and CNI-induced ED mice. Erectile function was measured through electrical stimulation of bilateral cavernous nerves, with subsequent intracavernous pressure parameter recordings. Mechanistic investigations included immunofluorescence staining, TUNEL assay, and western blot analysis. Additionally, ex vivo neurite outgrowth assays were conducted using dorsal root ganglia (DRG) and major pelvic ganglia (MPG) tissues.

Results: BMP2 expression was significantly decreased in the corpus cavernosum of CNI mice. Exogenous administration of recombinant BMP2 protein effectively enhanced erectile function in CNI mice, likely through the restoration of endothelial cells, smooth muscle cells, pericytes, and neuronal cells within the corpus cavernosum. Immunofluorescence staining and western blot analysis demonstrated that BMP2 treatment promoted angiogenesis by increasing endothelial cell proliferation and reducing apoptosis in the corpus cavernosum. Furthermore, ex vivo assays revealed that BMP2 promoted neurite sprouting in DRG and MPG tissues exposed to lipopolysaccharide. Mechanistic studies further indicated that BMP2 increased the expression of neurotrophic factors and VEGF, activating the AKT/eNOS signaling pathway.

Conclusion: The exogenous administration of recombinant BMP2 protein significantly improved erectile function in CNI mice, suggesting BMP2 as a promising therapeutic candidate for neurogenic ED.

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Silencing of IGFBP5 improves erectile function by inducing neurovascular regeneration in a cavernous nerve injury model

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Background/Aims: To investigate the therapeutic potential of eliminating IGFBP5 expression in improving erectile function in mice with cavernous nerve injury (CNI)-induced erectile dysfunction (ED).

Introduction: Radical prostatectomy for prostate cancer not only induces cavernous nerve injury but also causes cavernous angiopathy, which is responsible for poor responsiveness to phosphodiesterase-5 inhibitors. Insulin-like growth factor-binding protein 5 (IGFBP5) promotes cell death and induces apoptosis in various cell types.

Methods and Material: Eight-week-old male C57BL/6 mice were divided into four groups: a sham-operated group and three CNI-induced ED groups. The CNI-induced ED groups were treated with intracavernous injections 3 days before the CNI procedure. These injections included phosphate-buffered saline, scrambled control shRNA, or shRNA targeting mouse IGFBP5 lentiviral particles. One week after CNI, erectile function was evaluated and the penile tissue was then harvested for histological examination and western blot analysis. Additionally, the major pelvic ganglia (MPG) and dorsal root ganglia (DRG) were cultured for *ex vivo* neurite outgrowth assays.

Results: Following CNI, IGFBP5 expression in the cavernous tissues significantly increased, reaching its peak at day 7. *Ex vivo* studies demonstrated that ablation of IGFBP5 expression promotes neurite sprouting in MPG and DRG when exposed to lipopolysaccharide. *In vivo* studies demonstrated that ablating IGFBP5 expression in CNI-induced ED mice improved erectile function, likely owing to increased neurovascular contents, including endothelial cells, pericytes, and neuronal processes. Immunohistochemistry and western blot analysis revealed that ablating IGFBP5 expression in CNI-induced ED mice promoted neurovascular regeneration by increasing cell proliferation, reducing apoptosis, and decreasing Reactive oxygen species production. Additionally, western blot analysis demonstrated that IGFBP5 ablation attenuated the JNK/c-Jun signaling pathway, activated the PI3K/AKT signaling pathway, and increased VEGF and neurotrophic factor expression.

Conclusion: Ablating IGFBP5 expression enhanced neurovascular regeneration and ultimately improved erectile function in CNI-induced ED mice.

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Modified targetable tyrosine kinase inhibitors exert anti-angiogenic effects by inhibiting the VEGF-A induced VEGFR2 signaling in vitro

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Introduction: Ocular neovascularization-based diseases such as neovascular age-related macular degeneration (nAMD) and proliferative diabetic retinopathy (PDR) are among the leading causes of blindness. PDR and nAMD can be caused by abnormal blood vessel growth due to elevated levels of vascular endothelial growth factor (VEGF) in the eye. Here, we investigate the inhibitory potential of novel, chemically modified TKIs and hypothesize that a light-based approach enables targeting the molecules into specific tissues by permanently binding them to vascular endothelial growth factor receptor 2 (VEGFR2) and helps increase their inhibitory effect on neovascularization in vitro; thus, their concentration potentially could be reduced during oral administration.

Methods and Materials: In this study, the modified TKIs EYE1118 and EYE1090 were tested in the presence and absence of light in different assays. We performed ADP-Glo Kinase assay followed by Western Blot on recombinant VEGFR2 kinase. To determine the efficacy of the inhibitors in vitro, VEGFR2/NFAT reporter HEK293 cells were treated with the inhibitors (0.1 pM – 10 µM), and luciferase reporter assay was performed. Human retinal microvascular endothelial cells (HRMECs) were pre-incubated with compounds (0.1 nM - 10 µM) to test their inhibitory effect on in vitro angiogenesis.

Results: The kinase activity decreased significantly ($p<0.0001$) in the presence of the inhibitors in the dark, and the presence of light increased the inhibitory effect of EYE1118 ($p<0.01$) and EYE1090 ($p<0.001$) against recombinant VEGFR2 kinase even more. Both inhibitors strongly decreased the phosphorylation levels of Tyr951, Tyr1175, and Tyr1214 on recombinant VEGFR2 both with and without illumination. In HEK293 cells, VEGFR2 signaling was strongly inhibited by EYE1118 and EYE1090, and the inhibition increased significantly ($p<0.0001$) after illumination. Both molecules strongly inhibited the in vitro angiogenic potential of HRMECs seeded on extracellular matrix in the presence of light, and this inhibition was significantly more potent than in the dark.

Conclusion: Our results demonstrate that the new TKIs exhibit potent inhibitory effects both in vitro and in biochemical tests. Furthermore, it was confirmed that photoactivation enables them to be targeted and bound permanently to VEGFR2; thus, the inhibitory potential of these molecules can be increased when exposed to light.

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Endothelial cell adaptations to chemotherapy compromises barrier function and impacts whole tissue microenvironment

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Background/Aims: Microvascular EC (mvEC) are gatekeepers of organ function as first responders to tissue demands and circulating factors. Our previous research has demonstrated that the activation state of EC is a key determinant of metastatic success and that mvEC responses to environmental challenges are organ specific. Further investigation revealed that anthracyclines induce functional, molecular and metabolic reprogramming in ECs. As a cornerstone of cytotoxic therapy for aggressive, refractory, or metastatic cancers, anthracyclines play a crucial role in treatment strategies – because they are delivered intravenously, EC are the first cells to encounter, adapt and respond to this challenge. This, however, does not prevent or reverse metastatic progression. In this study, we explored how vascular remodelling in response to anthracyclines influence the tumour-free organs, and how this impacts whole tissue microenvironment, risk of distant relapse and compromised survorship.

Methods: Primary human and murine mvECs were treated with epirubicin at organ-specific IC50 concentrations. *In vivo* experiments involved mice receiving 10 mg/kg epirubicin. *In vitro* assays assessed critical EC functions such as barrier integrity, regenerative ability and secretory patterns.

Results: Our findings revealed that susceptibility to anthracycline-induced stress varies by organ, with brain ECs exhibiting an IC50 nearly three times higher than lung ECs. Surviving ECs that adapted to cytotoxic stress displayed impaired regenerative functions, including wound healing, proliferation, and angiogenesis, with recovery being dose-dependent. Notably, anthracyclines compromised EC barrier function. *Ex vivo* assays showed that anthracyclines disrupted VE-cadherin membrane localization, particularly in brain ECs, correlating with a marked reduction in HIF-2α activation—an essential regulator of VE-cadherin expression, subcellular localization, and blood-brain barrier stability. These observations were validated *in vivo*, where significantly fewer ECs were recovered from tumour-free organs in anthracycline-treated mice. Immunofluorescence and confocal microscopy showed reduced tight junction protein levels, and a transient increase in blood-brain barrier permeability. EC-derived inflammatory responses associated with barrier disruption varied between the brain and lung.

Conclusion: Anthracycline-based therapy significantly impacts microvascular integrity, EC behaviour, and EC-derived signalling in an organ-specific manner. Ongoing research aims to determine the long-term implications of these effects on organ function, organotropism, and metastatic risk.

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The role of LDLR and CB1R double knockout genotype on lipid-induced vascular wall remodeling in a mouse model of atherosclerosis

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Background/Aims: Atherosclerosis forms the background of several cardiovascular pathologies. LDL receptor knockout (LDLR-KO) mice kept on high-fat diet (HFD) develop high cholesterol levels and atherosclerosis (AS). In LDLR-KO mice fed HFD, vascular wall function deterioration is associated with structural lesions, calcified plaque formation and reduced vascular wall elasticity. Cannabinoid type 1 receptors (CB1Rs) induce acute vasodilation, although their contribution to cardiovascular pathologies is still controversial. Thus, we aimed to reveal the potential role of CB1Rs on vascular function and remodeling in AS-prone LDLR-KO mice.

Methods: Experiments were performed on LDLR and CB1R double knockout (KO) and wild type (WT) mice, kept on HFD or control diet (CD) for 5 months. Blood pressure was measured with tail cuff method during isoflurane anesthesia. Aorta samples were taken for further studies. Vascular functions of abdominal aorta rings were tested with wire myography. Vasorelaxation effects of acetylcholine (Ach, 1nM-1µM) were obtained after phenylephrine precontraction, were repeated with inhibitor of nitric oxide synthase (NOS), Nω-nitro-L-arginine. Serum cholesterol levels were also measured. Plaques were demonstrated on the aortic arch with oil red staining. Elastin density was detected with resorcin fuchsin staining. Immunostaining of endothelial NOS (eNOS) was obtained.

Results: Blood pressure was higher in LDLR-KO compared to WT mice (systolic/diastolic values: 110/84±5.8/6.8 vs. 102/80±3.3/2.5mmHg), which was significantly elevated to HFD (118/96±1.9/2 vs. 100/77±3.4/3.1mmHg, p<0,05) but attenuated in CB1R-KO HFD mice. HFD significantly elevated cholesterol levels in LDLR-KO mice, compared to WT (in average 1039±162 mg/dl vs. 91±18 mg/dl, p<0.05), also total plaque size was significantly increased (from 3.8±3 to 28.3±2%, p<0.05), these effects were not effectively influenced by the presence of CB1Rs. However, damaged Ach-induced relaxation in HFD animals was moderated in the CB1R-KO. HFD reduced elastin density in LDLR-KO mice (p<0.05), which was reversed in the absence of CB1R. Expression of eNOS was depressed in HFD WT mice compared to CD, which was augmented in CB1R-KO.

Conclusion: Our results indicate that the absence of CB1Rs significantly attenuates the vascular damage in AS. These results may contribute to new therapeutic options in cardiovascular disease by selectively modifying CB1R signaling.

Supported by the 2024-2.1.1-EKÖP-2024-00004 University Research Scholarship Programme of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund; PhD grants PhDKUT0561 (to Zsolt Vass) and PhDKUT0776 (to Kinga Shenker-Horváth) of the

Semmelweis University, Faculty of Health Sciences; and by the Hungarian Society of Hypertension, Research Grant 2023 (Mária Szekeres).

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Bioassay for vascular ischemia/reperfusion injury: mesenteric versus coronary arteries

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Introduction: For decades, research on the pathophysiological mechanisms of myocardial ischemia/reperfusion (I/R) injury has predominantly focused on cardiomyocytes. However, acute myocardial infarction also induce vascular injury, especially affecting the coronary microcirculation, which further exacerbate myocardial injury. In clinical studies, this vascular injury has been shown to be an important prognostic factor for patients with acute myocardial infarction independent of infarct size. Experimental studies on vascular I/R injury frequently rely on the bioassay of isolated mesenteric arteries.

Aim: We here aim to compare the vascular function of isolated rat mesenteric and coronary arteries in order to identify potential disparities that may exist in response to vascular I/R injury.

Methods and Material: Rat mesenteric and coronary arteries were isolated post mortem. All vessel segments were mounted on a wire small vessel myograph for isometric force measurements. The vessels were subjected to either simulated I/R (30 minutes hypoxia/10 minutes reoxygenation) or a corresponding time-matched control. Then, potassium chloride (KCl)-induced depolarization of the vascular smooth muscle cells was induced and maximal vasoconstriction to KCl quantified. Endothelium-dependent vasodilation was measured in response to carbachol (1×10^{-9} mol/L–1×10^{-4} mol/L) after pre-constriction with serotonin (3×10^{-6} mol/L).

Results: The maximal vasoconstriction in response to KCl and serotonin was comparable between time control and simulated I/R in both vessel types. With simulated I/R, the endothelium-dependent vasodilation was less pronounced, again, in both vessel types (in mesenteric arteries, at $1x10^{-7}$ mol/L carbachol (from 32.7±2.5 to 46.7±2.7% of preconstriction) and at $3x10^{-7}$ mol/L carbachol (form 7.7±2.5 to 21.5±2.7% of preconstriction), and in coronary arteries, from $1x10^{-6}$ (from 37.7±2.2 to 52.5±1.9% of preconstriction) to $1x10^{-4}$ (from 11.3±2.2 to 22.4±1.9% of preconstriction) mol/L carbachol).

Conclusion: Endothelium-dependent vasodilation is less pronounced in both vessel types after simulated I/R. Thus, in principle, both vessel types are useful for investigation of I/R-induced vascular injury mechanisms and evaluation of potential novel therapeutic strategies for vascular protection.

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PKC delta and pharmacomechanical coupling in the cerebral circulation: mechanisms to application

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Introduction: Constrictor stimuli set cerebral arterial blood flow through coupling mechanisms dependent on (electromechanical) and independent of (pharmacomechanical) membrane potential. Each elicit unique "vasomotor signatures", the latter (via protein kinase C, PKC) allowing small arterial segments to work independently of the broader network. What PKC isoforms drive pharmacomechanical coupling and if focal vasomotor behavior is observable in vivo are questions to be addressed herein.

Methods: A multiscale approach from cell-to-live brain, mice-to-humans and which includes western blotting, immunofluorescence, vessel myography, two photon fluorescence microscopy and computational modeling will be employed.

Results: A classic GPCR agonist, U46619, was first shown to drive a concentration dependent constriction, with electromechanical coupling preceding pharmacomechanical, the latter coupled to PKC. PKCδ, rather than PKCα mediated the pharmacomechanical response, irrespective of whether the agonist was superfused or applied by pipette to elicit focal constriction. This functional work aligned with cell analysis showing that PKCδ translocates to the membrane upon U46619 stimulation (indicative of activation) and western blot analysis noting that PKCδ blockade attenuated the phosphorylation of key pharmacomechanical regulators (CPI-17, HSP27). Pharmacomechanical coupling was observed in vivo (two photon microscopy) by monitoring the variance in diameter along a penetrating arteriole; this key measure of focal constriction attenuated diminished by PKCδ blockade. Realistic modeling of hemodynamic control revealed that focal constriction will markedly impact blood flow distribution among arteriolar branches.

Conclusion: PKCδ was identified as the key isoform mediating pharmacomechanical coupling and focal tone control in cerebral arteries. Discrete phamacomechanical control, as observed in live brain tissue, optimizes blood flow distribution among brain cortical layers. We theorize that augmented pharmacomechanical control underlies brain vascular pathobiology, including arterial vasospasm and transient ischemic attack.

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Subcellular mRNA localisation in endothelial barrier regulation

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Background/Aims: messenger (m)RNA localisation is a fundamental biological process that underpins a wide range of cellular functions. The aim of this work is to unveil how this mode of spatial control of gene expression modulates the behaviour of the endothelium.
Introduction: Upon transcription, mRNAs are localised to subcellular domains. Translation of localised mRNAs is believed to provide cost-effective benefits over the transport of the encoded proteins, to locally increase the concentration of "new" proteins and to support local protein complex formation. This ensures timely and regionalised cell responses to environmental stimuli that shape cell behaviour. In our current work, we hypothesize that spatial control of gene expression plays a role in modulating the endothelial cell-cell junctions and assist in the rapid architectural changes required for endothelial barrier remodelling.
Methods and Materials: We employ *in vitro* models of the endothelium in combination with imaging-based technologies that allow the visualisation of mRNAs and translation at subcellular resolution, including 10X spatial transcriptomics, single molecule FISH and puromycin incorporation.
Results: Initial observations suggested that RNA and newly synthesised proteins are present at endothelial cell-cell junctions. To understand the nature of the junctional RNAs, we carried out spatial transcriptomics. These experiments revealed how mRNAs encoding cytoskeletal modellers and endothelial barrier function colocalise to junctions. mRNA translation at these sites is not uniform and its inhibition reduces pore formation and barrier properties of endothelial monolayers.
Conclusion: mRNAs are targeted to endothelial cell-cell junctions and translation is required for the correct permeabilisation of the endothelium. We hope that our novel findings will contribute towards the development of innovative therapies to target endothelial dysfunction.

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