MOVD 2013
11th International Symposium on Mechanisms of Vasodilatation

4 – 6 October 2013
University Hospital Zurich
Dear Colleagues

It is a great pleasure to invite you to take part in the 11th International Symposium on Mechanisms of Vasodilatation which will take place in Zurich, Switzerland from Friday, October 4 to Sunday, October 6, 2013.

Symposia on Mechanisms of Vasodilatation have a long tradition and have established themselves as a premier event for vascular biologists from all over the world to get together to exchange and discuss exciting new research related to the control of the cardiovascular system in health and disease. The topics covered in this year’s meeting include nervous control of the cardiovascular system and novel ways to interact with it, in particular renal nerve ablation, the function of endothelial cells in the control of vasomotor tone as well as better understanding of the function of vascular smooth muscle cells and finally novel peptides that act in a paracrine fashion to control vascular tone.

This impressive tradition that started in Antwerp in the 1970ies will be continued in Zurich this year and we really hope that you will join us and participate in this interactive meeting as part of the exciting program.

Zurich is located in the heart of Europe and provides a mild and lovely climate in October as well as a city offering beautiful landscapes and an impressive culture that we hope to enjoy with you when visiting Switzerland this fall.

We sincerely hope that you will participate in this edition of Mechanisms of Vasodilatation and are looking forward to welcoming you in Zurich in October this year.

With best wishes and kindest regards,

Thomas F. Lüscher, MD, FRCP, Paul M. Vanhoutte, MD, PhD
Professor and Chairman of Cardiology    Chair Professor
University Hospital Zurich     Department of Pharmacology and Pharmacy
Congress President      Li Ka Shing Faculty of Medicine, Hong Kong
Congress Co-President
Welcome note

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Thomas F. Lüscher, MD, FRCP, Professor and Chairman of Cardiology University Hospital Zurich Congress President

Paul M. Vanhoutte, MD, PhD, Chair Professor Department of Pharmacology and Pharmacy Li Ka Shing Faculty of Medicine, Hong Kong Congress Co-President
International Scientific Committee

Chantal Boulanger, Ph.D., Paris FR
John C. Burnett, M.D., Rochester MN, USA
William B. Campbell, Ph.D., Milwaukee WI, USA
Richard A. Cohen, M.D., Boston MA, USA
Jo G.R. De Mey, Ph.D., Maastricht NL
Livius D’Uscio, Ph.D., Rochester MN, USA
Michel Féélou, Ph.D., Suresnes FR
Ingrid Fleming, Ph.D., Frankfurt a. M. DE
Yuansheng Gao, Ph.D., Beijing CN
Yu Huang, Ph.D., Hong Kong CN
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François Mach, M.D., Geneva CH
Thomas Münzel, M.D., Mainz DE
Ton Rabelink, M.D., Ph.D., Leiden NL
Valérie Schini-Kerth, Ph.D., Strasbourg FR
Hiroaki Shimokawa, M.D., Ph.D., Sendai JP
Stefano Taddei, M.D., Pisa IT
Paul M. Vanhoutte, M.D., Ph.D., Hong Kong CN/Riyadh SA
Johan van de Voorde, M.D., Ghent BE
Heikki Vapaatalo, M.D., Ph.D., Helsinki FI
Tony Verbeuren, Ph.D., Suresnes FR
Zhihong Yang, M.D., Fribourg CH

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Ulf Landmesser, M.D., Zurich
Christian Matter, M.D., Zurich
Frank T. Ruschitzka, M.D., Zurich
Isabella Sudano, M.D., Zurich
Felix C. Tanner, M.D., Zurich

Invited Lecturers

Stefan D. Anker, M.D., Berlin DE
Mario Bigazzi, M.D., Florence IT
Murray Esler, M.D., Melbourne AU
Uta Hoppe, M.D., Salzburg AT
Yu Huang, Ph.D., Hong Kong CN
Zvonimir S. Katusic, M.D. Rochester USA
Felix Mahfoud, M.D., Homburg/Saar DE
Marco Metra, M.D., Brescia IT
Ernesto L. Schiffrin, M.D., Ph.D., Montreal CA
Johannes-Peter Stasch, Ph.D., Wuppertal DE
Christian Templin, M.D., Zurich CH
Clinton R. Webb, M.D., Augusta USA
Oral Presenters

Takeshi Adachi, M.D., Tokorozawa JP
Alexander Akhmedov, Ph.D., Zurich CH
Cyril Auger, Ph.D., Illkirch FR
Jeremy Bellien, Ph.D., Rouen FR
Hidde Bult, Ph.D., Antwerp BE
William B. Campbell, Ph.D., Milwaukee USA
Keith M. Channon, M.D., Oxford UK
Richard A. Cohen, M.D. Boston USA
Jean-Luc Cracowski, Ph.D., Gernoble FR
Andreas Daiber, M.D., Ph.D., Mainz DE
Jo G.R. De Mey, Ph.D., Maastricht NL
Beate Fisslthaler, Ph.D., Frankfurt a. M. DE
Yuansheng Gao, Ph.D., Beijing CN
Kathryn M. Gauthier, Ph.D., Milwaukee USA
Shigeo Godo, Sendai JP
Pernille B.L. Hansen, Ph.D., Odense DK
Michael Hausding, Ph.D., Mainz DE
Ilkka Heinonen, Ph.D., Turku FI
Karin Kohlstedt, Ph.D., Frankfurt a. M. DE
Nicolle Kränkel, Ph.D., Zurich CH
Dae Hyun Lee, M.D., Ph.D., Leiden NL
Thomas F. Lüscher, M.D., Zurich CH
Virginia M. Miller, M.D., Rochester USA
Eduardo Nava, Ph.D., Albacete ES
Elena Osto, M.D., Ph.D., Zurich CH
Holger Schneider, Ph.D., Munich DE
Ulf Simonsen, M.D., Ph.D., Aarhus DK
Paul Stamm, Mainz DE
Isabella Sudano, M.D., Zurich CH
Stefano Taddei, M.D., Pisa IT
Xiaoyong Tong, M.D., Boston USA
Paul M. Vanhoutte, M.D., Ph.D., Hong Kong CN/Riyadh SA
Yu Wang, Ph.D., Hong Kong CN
Zhichao Zhou, Rotterdam NL
### Friday, 4 October 2013

**Grosser Hörsaal Ost**

#### Afternoon

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.15–13.30</td>
<td><strong>Welcome</strong></td>
</tr>
<tr>
<td></td>
<td>Paul M. Vanhoutte, Hong Kong / Saudi Arabia</td>
</tr>
<tr>
<td></td>
<td>Thomas F. Lüscher, Switzerland</td>
</tr>
<tr>
<td>13.30–15.00</td>
<td><strong>Symposium: Sympathetic Control of Renal Circulation and Blood Pressure.</strong></td>
</tr>
<tr>
<td></td>
<td>The Basis for Renal Nerve Ablation</td>
</tr>
<tr>
<td></td>
<td>Chairpersons: Tony Verbeuren, France and Thomas F. Lüscher, Switzerland</td>
</tr>
<tr>
<td>13.30–14.00</td>
<td><strong>Named Lecture</strong></td>
</tr>
<tr>
<td></td>
<td>8th John T. Shepherd Lecture on Nervous Control: Introduced by Richard A. Cohen</td>
</tr>
<tr>
<td></td>
<td>Renal nerves and the sympathetic control of arterial blood pressure</td>
</tr>
<tr>
<td></td>
<td>Murray Esler, Australia</td>
</tr>
<tr>
<td>14.00–14.20</td>
<td><strong>Renal nerve ablation with novel technologies: From catheters to balloons</strong></td>
</tr>
<tr>
<td></td>
<td>Uta Hoppe, Austria</td>
</tr>
<tr>
<td>14.20–14.40</td>
<td><strong>Renal vascular damage after renal nerve ablation: an OCT Study</strong></td>
</tr>
<tr>
<td></td>
<td>Christian Templin, Switzerland</td>
</tr>
<tr>
<td>14.40–15.00</td>
<td><strong>Clinical effectiveness of renal nerve ablation</strong></td>
</tr>
<tr>
<td></td>
<td>Felix Mahtoud, Germany</td>
</tr>
<tr>
<td>15.00–15.30</td>
<td><strong>Coffee Break</strong></td>
</tr>
<tr>
<td>15.30–17.30</td>
<td><strong>Oral Presentations: Endothelium-Dependent Dilatations</strong></td>
</tr>
<tr>
<td></td>
<td>Chairpersons: Valérie Schini-Kerth, France and Zhiihong Yang, Switzerland</td>
</tr>
<tr>
<td>15.30–15.45</td>
<td>Inducible endothelium-derived hyperpolarizing factor: Role of the 15-lipoxygenase pathway</td>
</tr>
<tr>
<td></td>
<td>William B. Campbell, USA</td>
</tr>
<tr>
<td>15.45–16.00</td>
<td>Importance of physiological balance between nitric oxide and EDHF in endothelium-dependent vasodilatation</td>
</tr>
<tr>
<td></td>
<td>Shigeo Godo, Japan</td>
</tr>
<tr>
<td>16.00–16.15</td>
<td>Differences between basal and stimulated nitric oxide activity in the mouse aorta in relation to endothelial nitric oxide synthase uncoupling</td>
</tr>
<tr>
<td></td>
<td>Hidde Bult, Belgium</td>
</tr>
<tr>
<td>16.15–16.30</td>
<td>T-type voltage-gated calcium channels (Cav3.1) are co-localized with eNOS and promote vasodilatation during depolarization in mesenteric arteries</td>
</tr>
<tr>
<td></td>
<td>Pernille B.L. Hansen, Denmark</td>
</tr>
<tr>
<td></td>
<td>Paul Stamm, Germany</td>
</tr>
<tr>
<td>16.45–17.00</td>
<td>Altered coronary endothelial signaling in response to uridine adenosine tetraphosphate in diabetes</td>
</tr>
<tr>
<td></td>
<td>Zhichao Zhou, Netherlands</td>
</tr>
<tr>
<td>17.00–17.15</td>
<td>5’adenosine monophosphate activated protein kinase (AMPK) induces vasodilatation of microvessels by reducing smooth muscle calcium sensitivity</td>
</tr>
<tr>
<td></td>
<td>Holger Schneider, Germany</td>
</tr>
<tr>
<td>17.15–17.30</td>
<td>Polyphenol-rich blackcurrant juice induces NO-mediated relaxation in porcine coronary artery rings via a copper- and iron-dependent redox-sensitive activation of the Src/PI3-kinase/Akt/eNOS pathway</td>
</tr>
<tr>
<td></td>
<td>Cyril Auger, France</td>
</tr>
</tbody>
</table>
Program

Friday, 4 October 2013
Afternoon

17.30–19.00  Wine & Cheese Session  Foyer Dick & Davy
             Poster presentations

19.00–19.30  Named Lecture  Grosser Hörsaal Ost
             7th Robert F. Furchgott Lecture on Endothelium: Introduction by William B. Campbell
             PPAR agonists preserve endothelial function by taking different routes
             Yu Huang, China

19.45–20.30  Piano Recital  Pascal Sigrist, Belgium

Saturday, 5 October 2013  Grösser Hörsaal Ost
Morning

08.00–09.00  Symposium: Novel Vasoactive Peptides
             Chairpersons: Zvonimir S. Katusic, USA and Frank T. Ruschitzka, Switzerland

08.00–08.20  Relaxin, a pregnancy hormone in control of the circulation
             Mario Bigazzi, Italy

08.20–08.40  Serelaxin, a novel treatment for acute heart failure
             Marco Metra, Italy

08.40–09.00  Ularitide, a new peptide for the treatment of heart failure?
             Stefan D. Anker, Germany

09.00–09.30  Named Lecture
             6th David F. Bohr Lecture on Vascular Smooth Muscle: Introduced by Virginia Miller
             Mitochondria-derived peptides: Novel mediators of vasodilatation
             Clinton R. Webb, USA

09.30–10.00  Coffee Break
Program

Saturday, 5 October 2013
Grosser Hörsaal Ost

Morning

10.00–11.45  Oral Presentations: Endothelial Function
Chairpersons: Ingrid Fleming, Germany and Giovanni Camici, Switzerland

10.00–10.15  Glutathione adducts on specific cysteine thiols of endothelial cell proteins regulate ischemic blood flow recovery
Richard A. Cohen, USA

10.15–10.30  Angiotensin converting enzyme inhibition enhances bradykinin relaxations through nitric oxide and B1 receptor activation in bovine coronary arteries
Kathryn M. Gauthier, USA

10.30–10.45  Deamidated lipocalin-2 induces endothelial dysfunction in dietary obese mice
Yu Wang, China

10.45–11.00  AMP-activated kinase alpha1 subunit in endothelial cells regulates vascular reactivity via eNOS phosphorylation on THR495
Beate Fisslthaler, Germany

11.00–11.15  Roux-en-Y gastric bypass immediately improves endothelial dysfunction and HDL properties by a glucagon like peptide-1 mediated body weight loss-independent effect
Elena Osto, Switzerland

11.15–11.30  Perivascular visceral fat causes endothelial dysfunction of mesenteric resistance arteries in a rat model of metabolic syndrome (SHROB). Role of prostaglandins
Eduardo Nava, Spain

11.30–11.45  Mechanisms underlying the vasodilator effects of cystamine in small mesenteric arteries
Ulf Simonsen, Denmark

11.45–13.00  Lunch
Saturday, 5 October 2013

**Grosser Hörsaal Ost**

**Afternoon**

**13.00–13.30**

**Named Lecture**

5th Björn Folkow Lecture on Growth and Remodeling: Introduced by Jo G.R. De Mey

**Effects of immune mechanisms on the vasculature in hypertension**

Ernesto L. Schiffrin, Canada

**13.30–14.30**

**Symposium: Endothelin-1 and Vascular Control**

Chairpersons: Michel Féletou, France and Ton Rabelink, Netherlands

**13.30–14.00**

**Endothelin-1 and vascular tone**

Thomas F. Lüscher, Switzerland

**14.00–14.15**

**Negative allosteric modulation of endothelin ETA receptor function in resistance arteries**

Jo G.R. De Mey, Netherlands

**14.15–14.30**

**Low-grade inflammation participates in the enhanced vasoconstriction to endogenous endothelin-1 in small vessels from obese patients**

Stefano Taddei, Italy

**14.30–15.45**

**Oral Presentations: Vasodilatation in the Human**

Chairpersons: Stefano Taddei, Italy and Christian Matter, Switzerland

**14.30–14.45**

**Stress-induced vascular dysfunction: Evaluation of sympathetic activity and endothelial function in patients with Takosubo Syndrome**

Isabella Sudano, Switzerland

**14.45–15.00**

**Endothelial glycocalyx accessibility determines microvascular perfusion and metabolic balance in obesity**

Dae Hyun Lee, Netherlands

**15.00–15.15**

**Effects of menopausal hormone treatments on platelet ATP secretion and vasoactive prostanoids in recently menopausal women**

Virginia M. Miller, USA

**15.15–15.30**

**The regulation of bone blood flow at rest and during exercise in humans**

Ilkka Heinonen, Finland

**15.30–15.45**

**Involvemnt of cytochrome epoxygenase metabolites in cutaneous postocclusive hyperemia in humans**

Jean-Luc Cracowski, France

**15.45–16.15**

**Coffee Break**

**16.15–17.30**

**Poster Presentations**

Foyer Dick & Davy

**17.30**

**Transfer to the Official Dinner**

Schloss Laufen by the Rhine Falls
Sunday, 6 October 2013

**Grosser Hörsaal Ost**

**Morning**

08.30–09.50 **Symposium: Nitric Oxide and Soluble Guanylyl Cyclase**
Chairpersons: Hiroaki Shimokawa, Japan and Francesco Cosentino, Switzerland

08.30–08.45 **Endothelium-specific deletion of GCH1 reveals cell-autonomous requirements for endothelial tetrahydrobiopterin in regulation of nitric oxide vs. hydrogen peroxide-mediated vasodilatation and blood pressure**
Keith M. Channon, England

08.45–09.00 **Bioactivation and tolerance to nitrates**
Andreas Daiber, Germany

09.00–09.15 **Stimulators of soluble guanylate cyclase for the treatment of cardiopulmonary diseases**
Johannes-Peter Stasch, Germany

09.15–09.30 **Vasoconstriction by activation of soluble guanylyl cyclase**
Paul M. Vanhoutte, Hong Kong/Saudi Arabia

09.30–09.50 **Cyclic IMP and hypoxic contraction**
Yuansheng Gao, China

09.50–10.15 **Coffee Break**

10.15–10.45 **Named Lecture**
4th Paul M. Vanhoutte Lecture on Vascular Pathology: Introduced by Hiroaki Shimokawa

**Endothelial nitric oxide in cerebrovascular disease**
Zvonimir S. Katusic, USA

10.45–12.45 **Oral Presentations: Senescence and Inflammation**
Chairpersons: Richard A. Cohen, USA and Felix C. Tanner, Switzerland

10.45–11.00 **Endothelial overexpression of LOX-1 decreases arterial thrombosis and TF expression in vivo**
Alexander Akhmedov, Switzerland

11.00–11.15 **The roles of smooth muscle NOX4 in atherosclerosis and restenosis**
Xiaoyong Tong, USA

11.15–11.30 **Deletion of hepatic ERK2 decreased the SERCA2 expressions, which can account for vascular oxidative stress and endothelial dysfunction in metabolic stress**
Takeshi Adachi, Japan

11.30–11.45 **Endothelial angiotensin converting enzyme (ACE) levels decrease in response to activation of the AMP-activated protein kinase (AMPK) via P53 and micro-RNA (miR)-143/145**
Karin Kohlstedt, Germany

11.45–12.00 **CD40 ligand promotes angiotensin-II induced vascular inflammation, thrombin sensitivity, oxidative stress and endothelial dysfunction**
Michael Hausding, Germany

12.00–12.15 **The role of miR-483-3P in endothelial homeostasis and response to vascular injury**
Nicolle Kränkel, Switzerland

12.15–12.30 **Cardiovascular impact of soluble epoxide hydrolase inhibition in a murine model of type 2 diabetes**
Jeremy Bellien, France

12.30–12.45 **Closing Remarks**
Thomas F. Lüscher, Switzerland

12.45 **Sandwich Lunch**
# Table of Content Oral Presentations

## Endothelium-Dependent Dilatations

- **p. 12–16**
  1. William B. Campbell, Ph.D., Milwaukee USA
  2. Shigeo Godo, Sendai JP
  3. Hidde Bult, Ph.D., Antwerp BE
  4. Pernille B.L. Hansen, Ph.D., Odense DK
  5. Paul Stamm, Mainz DE
  6. Zhichao Zhou, Rotterdam NL
  7. Holger Schneider, Ph.D., Munich DE
  8. Cyril Auger, Ph.D., Illkirch FR

## Endothelial Function

- **p. 17–20**
  1. Richard A. Cohen, M.D. Boston USA
  2. Kathryn M. Gauthier, Ph.D., Milwaukee USA
  3. Yu Wang, Ph.D., Hong Kong CN
  4. Beate Fisslthaler, Ph.D., Frankfurt a. M. DE
  5. Elena Osto, M.D., Ph.D., Zurich CH
  6. Eduardo Nava, Ph.D., Albacete ES
  7. Ulf Simonsen, M.D., Ph.D., Aarhus DK
  8. Stephen T. O’Rourke, Ph.D., Fargo USA

## Endothelin-1 and Vascular Control

- **p. 21**
  1. Jo G.R. De Mey, Ph.D., Maastricht NL
  2. Stefano Taddei, M.D., Pisa IT

## Vasodilatation in the Human

- **p. 22–24**
  1. Isabella Sudano, M.D., Zurich CH
  2. Dae Hyun Lee, M.D., Ph.D., Leiden NL
  3. Virginia M. Miller, M.D., Rochester USA
  4. Ilkka Heinonen, Ph.D., Turku FI
  5. Jean-Luc Cracowski, Ph.D., Gernoble FR

## Nitric Oxide and Soluble Guanylyl Cyclase

- **p. 25–26**
  1. Keith M. Channon, M.D., Oxford UK
  2. Andreas Daiber, M.D., Ph.D., Mainz DE
  3. Yuansheng Gao, Ph.D., Beijing CN

## Senescence and Inflammation

- **p. 27–30**
  1. Alexander Akhmedov, Ph.D., Zurich CH
  2. Xiaoyong Tong, M.D., Boston USA
  3. Takeshi Adachi, M.D., Tokorozawa JP
  5. Michael Hausding, Ph.D., Mainz DE
  6. Nicolle Kränkel, Ph.D., Zurich CH
  7. Jeremy Bellien, Ph.D., Rouen FR
INDUCIBLE ENDOTHELIUM-DERIVED HYPERPOLARIZING FACTOR: ROLE OF THE 15-LIPOXYGENASE PATHWAY

William B. CAMPBELL and Kathryn M. GAUTHIER

Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee WI, U.S.A. 53226

Introduction: Endothelium-derived hyperpolarizing factors (EDHFs) regulate vascular tone by contributing to the vasorelaxations to shear stress and endothelial agonists such as bradykinin and acetylcholine. 15(S)-Hydroxy-11,12-epoxyeicosatrienoic acid (15-H-E12,14-EET) and 11(R),12(S),15(S)-trihydroxyeicosatrienoic acid (11,12,15-THETA) are endothelial metabolites of the 15-lipoxygenase (15-LO) pathway of arachidonic acid (AA) metabolism and are EDHFs. 11,12,15-THETA activates smooth muscle cell small conductance, calcium-activated potassium channels causing membrane hyperpolarization and relaxation. Regulation of 15-LO expression is by transcriptional, translational and epigenetic mechanisms. Hypoxia, aging, hypercholesterolemia, atherosclerosis, anemia, estrogen, interleukins and possibly other hormones increase 15-LO expression. We hypothesize that increased 15-LO-1 expression increases AA metabolism to vasoactive metabolites and increases EDHF-dependent vascular relaxation.

Methods and Results: Western immunoblot revealed increased 15-LO-1 expression in arteries from male rabbits fed a high cholesterol diet or exposed to hypoxia and in arteries exposed to interleukin-13 or estrogen compared to normal rabbits. These expression profiles correlate with increased 15-LO-mediated AA metabolism. The increase in endothelial 15-LO from hypoxia, interleukin-13 or hypercholesterolemia resulted in increased synthesis of 15-H12,14-EET, 11,12,15-THETA and 15-HETE. In vascular myograph studies, acetylcholine and AA relaxations were significantly increased in arteries from hypoxic rabbits and rabbits fed a high cholesterol diet and arteries exposed to interleukin-13 compared to arteries of normal rabbits. Additionally, vascular smooth muscle electrode impalement showed increased AA-induced membrane hyperpolarization in arteries from the hypoxic or high cholesterol rabbits compared normal rabbits.

Conclusion: Endothelial cell expression of 15-LO regulates activity of the 15-LO/15-H12,14-EET/11,12,15-THETA pathway and its contribution to vascular tone. Thus, the 15-LO pathway represents the first example of an inducible EDHF. In addition to 15-LO metabolites, a number of chemicals have been identified as EDHFs and their contributions to vascular tone vary with species and vascular bed. The reason for multiple EDHFs has evaded explanation. However, EDHFs functioning as constitutive EDHFs or inducible EDHFs may explain the need for chemically and biochemically distinct pathways for EDHF activity and the variation in EDHFs between species and vascular beds. This new EDHF classification provides a framework for understanding EDHF activity in physiological and pathological conditions.

IMPORTANCE OF PHYSIOLOGICAL BALANCE BETWEEN NITRIC OXIDE AND EDHF IN ENDOTHELIUM-DEPENDENT VASODILATATION

Shigeo GODO, Hiroki SAITO, Ayuko SAWADA, Budbazar ENKHJARGAL, Hiroaki SHIMOKAWA

Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan

Introduction: Vascular tone is finely modulated by endothelium-derived relaxing factors (EDRFs), including prostacyclin (PGI2), nitric oxide (NO) and endothelium-derived hyperpolarizing factor(s) (EDHF). The contribution of these EDRFs to endothelium-dependent vasodilatations varies depending on the size of blood vessels; large conduit arteries are predominantly regulated by NO and small resistance arteries by EDHF. We have previously demonstrated that hydrogen peroxide (H2O2) derived from endothelial NO synthase (eNOS) is an EDHF in animals and humans. Furthermore, we have recently demonstrated that caveolin-1 (Cav-1), which functionally inhibits eNOS, is substantially involved in the functional inhibition of eNOS in resistance vessels in mice. In the present study, we thus examined how the balance between NO and EDHF is important in understanding EDHF activity in physiological and pathological conditions.

Method: Male wild-type (WT), Cav-1-KO and eNOS-Tg mice were used. Systolic blood pressure was measured by tail-cuff method. Isometric tensions and membrane potentials of the aortae and small mesenteric arteries were recorded in organ chamber experiments and by microelectrode method, respectively. Coronary flow response to bradykinin was examined in the Langendorff-perfused heart experiment. In all experiments, EDHF-mediated responses were examined in the presence of indomethacin (10 microM) and N’omega-nitro-L-arginine (L-NNA, 100 microM).

Results: Systolic blood pressure tended to be lower in Cav-1-KO as compared with WT mice (WT 110±3 vs. Cav-1-KO 105±3 mmHg, P=0.29) and was significantly lower in eNOS-Tg mice (eNOS-Tg 83±1 mmHg, P<0.05 vs. WT). In small mesenteric arteries from both Cav-1-KO and eNOS-Tg mice, NO-mediated relaxations were significantly enhanced as expected, whereas EDHF-mediated responses were markedly impaired (-logEC50 mol/L, WT 7.31±0.09 vs. Cav-1-KO 6.81±0.10, eNOS-Tg 6.91±0.08, both P<0.05; maximum % relaxation, WT 90.6±1.9 vs. Cav-1-KO 40.8±14.1, eNOS-Tg 51.7±7.1, both P<0.05). In membrane potential recordings, Cav-1-KO mice showed attenuated hyperpolarization responses, while eNOS-Tg mice had less negative resting potentials as compared with WT. Langendorff experiments also showed that EDHF-mediated coronary flow responses were significantly impaired in both Cav-1-KO and eNOS-Tg mice (%increase in coronary flow; WT 113.9±10.3, vs. Cav-1-KO 51.3±12.9, eNOS-Tg 31.6±5.5, both P<0.01). Cav-1-KO mice showed cardiac hypertrophy with 25% greater heart weight as compared with WT (WT 120±12 vs. Cav-1-KO 146±16 mg, P<0.05).

Conclusion: These results indicate that excessive activation of eNOS disrupts the physiological balance between NO and EDHF, resulting in impaired cardiovascular homeostasis.
Oral Presentations

Endothelium-Dependent Dilatations

3

DIFFERENCES BETWEEN BASAL AND STIMULATED NITRIC OXIDE ACTIVITY IN MOUSE AORTA IN RELATION TO ENDOTHELIAL NITRIC OXIDE SYNTHASE UNCOUPLING

Johanna T.H. VÆRN LANGEN1, Cor E. VAN HOVE1, Paul FRANSEN2, Hidde BULT1

1Division of Pharmacology, University of Antwerp, Antwerp-Wilrijk, Belgium
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Introduction: We recently showed that the mouse aorta displays a huge basal endothelial nitric oxide (NO) synthase (eNOS) activity that quickly subsides in an organ bath, while acetylcholine-(ACH)-induced relaxation is maintained. In this study we investigated the role of eNOS uncoupling, the effects of L-arginine, N-methyl-L-arginine (L-NMMA) and N,N-dimethylarginine (ADMA) and regional differences within the aorta on basal and ACh-induced eNOS activity.

Method: Aortic rings (1.5 mm) of C57/B6 mice were mounted in organ baths containing Krebs' solution. Exactly 1 h and 4 h after isolation basal NO was assessed by its ability to suppress phenylephrine (PE, 1 microM)-induced isometric force. After stabilisation of the PE response increasing ACh (0.003-10 microM) concentrations were given. Catalase (2400 U/ml) was added 30 min prior to PE; other compounds were added 15 min before PE, and were continuously present thereafter. To compare aortic regions, PE contractions were only performed at 1 h followed by ACh concentrations, after which catalase was added to determine the contribution of hydrogen peroxide to the relaxation. At the end of each experiment a PE contraction was performed in the presence of 300 microM N-nitro-L-arginine and 300 microM N-nitro-L-arginine methyl ester. Immunoblotting was used to investigate Akt and eNOS phosphorylation.

Results: PE-induced force increased between 1 h and 4 h after aorta isolation, while ACh relaxation remained unaltered. Catalase had no effect on PE contractions or ACh relaxation at 1 h, but greatly inhibited ACh relaxation at 4 h, pointing to eNOS uncoupling. The effect of catalase correlated inversely with basal NO availability. The time-dependent changes in eNOS activity coincided with attenuation of AktSer473 and eNOSSer1177 phosphorylation. Treatment with superoxide dismutase (100 U/ml) or 1mM L-arginine prevented eNOS uncoupling at 4 h, but failed to influence the loss of basal NO. L-NMMA and ADMA greatly inhibited basal eNOS activity with only partial or no suppression of ACh relaxation, respectively, and ADMA induced some uncoupling of ACh-induced eNOS activity. The ascending aorta displayed less basal NO release, but highly increased sensitivity for ACh compared to the central descending aorta. On the contrary, the aortic arch displayed higher basal NO production, but greatly attenuated ACh-induced relaxation that was largely inhibited by catalase.

Conclusion: After aorta isolation basal NO formation declined in parallel with uncoupling of ACh-induced eNOS activity, whereby hydrogen peroxide formation maintained a seemingly normal relaxation. Both processes were, however, not directly related since SOD and L-arginine treatment prevented eNOS uncoupling, but failed to sustain basal NO activity. This suggests that only receptor-stimulated eNOS became uncoupled, or that uncoupled eNOS lost its constitutive activity, but produced hydrogen peroxide upon ACh stimulation. Moreover, the differences between basal eNOS and stimulated eNOS activity may be of (patho)physiological relevance as demonstrated by the differential effects of the endogenous NO inhibitors L-NMMA and ADMA, and the striking regional differences in freshly isolated aortas.

4

T-TYPE VOLTAGE-GATED CALCIUM CHANNELS (Cav3.1) ARE CO-LOCALIZED WITH eNOS AND PROMOTE VASODILATATION DURING DEPOLARIZATION IN MESENTERIC ARTERIES.

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Introduction: Voltage-gated calcium channels of the T-type (Cav3.2) are essential for the acetylcholine-induced relaxation of coronary arteries and involved in dilatation of renal blood vessels. We investigated the T-type channel-dependent dilatation in Wt and Cav3.1 knock-out mice.

Method: Changes in luminal diameter in isolated perfused mesenteric arteries were determined. Immuno-precipitation and staining were performed for eNOS and Cav3.1. Mean arterial blood pressure and heart rate were measured in conscious mice through chronic indwelling catheters before and after infusion of L-NAME

Results: The secondary dilatation after potassium-induced contraction was reduced significantly in Cav3.1–/- compared to Wt mice and use of eNOS -/- mice led to a significantly attenuated dilatation. Immunoprecipitation of Cav3.1 pulled down eNOS, and precipitation of eNOS pulled down Cav3.1 showing co-localization of Cav3.1 and eNOS. Confocal laser-scanning microscopy of mesenteric arteries labeled with antibodies against eNOS and Ca.3.1 confirmed the co-localization of eNOS and Ca.3.1 in endothelial cells, and showed that Cav3.1 was also expressed in vascular smooth muscle cells. NOS inhibition elicited a significant increase in blood pressure that was sustained after four days in Wt mice (12±2%) whereas no increase was observed in Cav3.1-KO.

Conclusion: Endothelial calcium-influx through Cav3.1 T-type calcium channels that are co-localized with eNOS, stimulates eNOS, and thereby affects NO-dependent dilatation after depolarization in mesenteric resistance arteries. Our findings in vivo suggest that this mechanism is important for maintaining the systemic tone of NO on the total peripheral resistance.
GLUTATHIONE PEROXIDASE-1-DEFICIENCY POTENTIATES DYSREGULATORY MODIFICATIONS OF ENDOTHELIAL NITRIC OXIDE SYNTHASE AND VASCULAR DYSFUNCTION IN AGING

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Recently, we demonstrated that gene ablation of mitochondrial manganese superoxide dismutase and aldehyde dehydrogenase-2 markedly contributed to age-related vascular dysfunction and mitochondrial oxidative stress. The present study sought to investigate the extent of vascular dysfunction and oxidant formation in glutathione peroxidase-1 deficient (GPx-1−/−) mice during the aging process with special emphasis on dysregulation (uncoupling) of the endothelial nitric oxide synthase (eNOS). GPx-1−/− mice on a C57BL/6 background at 2, 6, and 12 months of age were used. Vascular function was significantly impaired in 12 months old GPx-1−/− mice as compared to age-matched controls. Oxidant formation, detected by 3-nitrotyrosine staining and dihydroethidine-based fluorescence microtopography, was increased in the aged GPx-1−/− mice. Aging per se caused a substantial protein kinase C- and protein tyrosine kinase-dependent phosphorylation as well as S-glutathionylation of eNOS causing uncoupling, a phenomenon that was more pronounced in aged GPx-1−/− mice. GPx-1 ablation increased adhesion of leukocytes to cultured endothelial cells and CD68 staining in cardiac tissue. Aged GPx-1−/− mice displayed increased oxidant-formation as compared to their wild type littermates, triggering redox-signaling pathways associated with eNOS dysfunction and uncoupling.

Thus our data demonstrate that aging leads to decreased nitric oxide bioavailability due to eNOS dysfunction and uncoupling of the enzyme leading to endothelial dysfunction, vascular remodeling, and promotion of adhesion and infiltration of leukocytes into cardiovascular tissue, all of which was more prominent in aged GPx-1−/− mice.
ALTERED CORONARY ENDOTHELIAL SIGNALING IN RESPONSE TO URIDINE ADENOSINE TETRAPHOSPHATE IN DIABETES

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Introduction: Uridine adenosine tetraphosphate (Up4A) has been recently been identified as a novel endothelium-derived vasoactive factor, which produces potent vasoconstriction via purinergic P2X and P2Y receptors. In contrast to the vasoconstrictor effect by Up4A in most arterial beds of rodent, we recently demonstrated that Up4A produces a potent vasodilator effect in the healthy porcine coronary microcirculation. However, the coronary vasomotor responses to Up4A in diseased conditions, such as diabetes mellitus (DM), have not been explored to date. Here we investigated the vasomotor responses to Up4A in isolated coronary small arteries from diabetic swine and studied the contribution of different endothelium-derived relaxing and/or contracting factors (EDRF/EDCF) as well as the involvement of purinergic receptor subtypes.

Method: Coronary small arteries (~150 µm), dissected from the apex of healthy swine and swine 6 months after induction of type 2 DM with streptozotocin (150 mg/kg, i.v.) and fed a high fat diet, were mounted on wire myographs for functional studies. Purinergic receptor expression was assessed by real-time PCR.

Results: DM swine showed elevated plasma levels of glucose, HDL, LDL and triglyceride. Although Up4A (10^-9-10^-5 µM) produced similarly potent vasodilation in control and DM swine, endothelial denudation as well as eNOS inhibition attenuated the response to Up4A in control but not in DM swine, indicative of endothelial dysfunction in DM swine (Fig. 1A, B/1E, F). Combined blockade of NOS and cyclooxygenase (COX) had no effect on Up4A-induced vasodilation as compared to NOS blockade alone in control, but enhanced Up4A-induced vasodilation in DM (Fig. 1B, F). This effect of COX-inhibition was mimicked by selective thromboxane synthase inhibition (Fig. 1D, H). Cyclomephamine (P450 (CYP 2C9) metabolites exerted a vasoconstrictor influence in response to Up4A in control, but served as a vasodilator in DM (Fig. 1C, G). Finally, purinergic P2X7 receptor blockade attenuated Up4A-induced vasodilation in control but had no effect in DM, while P2Y1 and P2Y6 blockade had no effect in control but significantly attenuated Up4A-induced vasodilation in DM. mRNA of P2X1, P2X7, P2Y1, P2Y2, P2Y4 and P2Y6 was present in the endothelium-intact coronary small arteries with a lower expression for P2X7 in DM as compared to control.

Conclusion: Up4A produces a potent coronary vasodilation in DM, which is as potent as that in control. Despite the maintained vasodilation in DM, marked alterations occurred in the involvement of EDCFs and EDRFs that may be mediated by differential expression and activation of different purinergic receptor subtypes.

Fig. 1. LNAME: NOS inhibitor; Indo: COX inhibitor; Sulfa: CYP 2C9 inhibitor; Ozagrel: thromboxane synthase inhibitor. Values are mean±SEM. *P<0.05 vs Control; †P<0.05 effect of Sulfa; %P<0.05 effect of LNAME+Ozagrel in DM differs from that in Control; $P<0.05 effect of LNAME+Ozagrel in DM differs from LNAME+Indo in DM.
Oral Presentations

Endothelium-Dependent Dilatations

7

5' ADENOSINE MONOPHOSPHATE ACTIVATED PROTEIN KINASE (AMPK) INDUCES VASODILATION OF MICROVESSELS BY REDUCING SMOOTH MUSCLE CALCIUM SENSITIVITY

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Introduction: The heterotrimeric enzyme AMP-activated protein kinase (AMPK) plays a central role in the regulation of energy homeostasis at both the cellular and whole body level. The manifold effects of AMPK activation also comprise vasodilation. However, the cellular mechanisms of action of AMPK in microvessels have not yet been clarified. We studied whether AMPK could alter the calcium sensitivity of the contractile apparatus.

Method: Isolated small murine mesenteric arteries were mounted in a pressure myograph system. Vascular diameter was registered by videomicroscopy and smooth muscle intracellular calcium concentration ([Ca2+]i) by Fura 2 fluorometry. AMPK was activated pharmacologically by adding A769662 (A76) or PT-1 to the organ bath.

Conclusion: Activation of each AMPK alpha-subunit to calcium sensitivity was tested by studying vessels of alpha_1- and alpha_2- subunit knockout mice. For protein extraction, vessels were rapidly frozen in trichloroacetic acid (TCA). Influences of AMPK activation on levels of phospho-MLC_20 (myosin light chain) were assessed by Western Blot technique.

Results: A76 as well as PT-1 induced dose dependent and endothelium independent vasodilation of vessels preconstricted by elevation of extracellular K+ (60 mM). This dilatation was not associated with significant decreases of [Ca2+]i. The stepwise increase of [Ca2+]i (by elevating the extracellular calcium concentration from zero to 3 mM in depolarized vessels) induced corresponding increases of vascular tone. A76 or PT-1 did not alter the increase in [Ca2+]i but significantly reduced the accompanying vasoconstriction indicating a reduced sensitivity of the contractile apparatus to [Ca2+]i. Incubation of microvessels with the MLCP inhibitor Calyculin A (100 nM) completely blocked the dilator effect of A76 or PT-1.

Conclusion: Activation of one AMPK alpha-subunit altered calcium sensitivity. Furthermore, these findings indicate that a mutual compensatory role of the alpha-subunits is possible in the control of calcium desensitization.

8

POLYPHEONOL-RICH BLACKCURRANT JUICE INDUCES NO-MEDIATED RELAXATION IN PORCINE CORONARY ARTERY RINGS VIA A COPPER- AND IRON-DEPENDENT REDOX-SENSITIVE ACTIVATION OF THE SRC/PI3-KINASE/aktu/ENOS PATHWAY

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Introduction: Numerous epidemiological and clinical studies indicate that consumption of polyphenol-rich food and beverages is associated with a protective effect on the cardiovascular system. The beneficial effect has been attributed, at least in part, to the improvement of the vascular function.

Indeed, polyphenols have been reported to be potent inducers of two major endothelial vasoprotective mechanisms, the formation of nitric oxide (NO) and the induction of endothelium-derived hyperpolarization (EDH). The aim of the present study is to determine whether a polyphenol-rich blackcurrant juice (2.7 g/l) improves the vasoprotective endothelial function, and, if so, to characterize the underlying mechanism.

Methods: The reactivity of porcine coronary artery rings was assessed in organ chambers, and the expression and phosphorylation levels of proteins in cultured porcine coronary endothelial cells by Western blot analysis.

Results: Polyphenol-rich blackcurrant juice caused potent relaxations in coronary artery rings with endothelium but not in those without endothelium. Relaxations to blackcurrant juice were significantly reduced by an eNOS inhibitor, not affected by inhibition of endothelium-dependent hyperpolarization, and abolished by both treatments. Blackcurrant juice-induced NO-mediated relaxations were significantly reduced by membrane permeant analogues of superoxide dismutase and catalase, inhibitors of either Src or PI3-kinase, and by calmidazolium, a calmodulin inhibitor. The NO-mediated relaxation was not affected by inhibitors of either PKC, EGFR, IGF, or of several endogenous enzymes involved in the formation of ROS (NADPH oxidase, xanthine oxidase, mitochondrial respiration chain, cytochrome P450), but was significantly reduced by chelators of either copper or iron. In cultured porcine coronary artery endothelial cells, blackcurrant juice increased the formation of NO as assessed by electron paramagnetic resonance spectroscopy. Moreover, blackcurrant juice induced the phosphorylation of Akt and eNOS on activator sites and these phosphorylation were inhibited membrane permeant analogues of superoxide dismutase and catalase and inhibitors of either Src or PI3-kinase.

Conclusion: Blackcurrant juice is a potent inducer of endothelium-dependent NO-mediated relaxations in porcine coronary artery rings. The NO-mediated relaxation involves an intracellular copper- and iron-dependent redox-sensitive activation the Src/PI3-kinase/Akt pathway leading to activation of eNOS by phosphorylation at Ser 1177 and subsequent formation of NO.
GLUTATHIONE ADDUCTS ON SPECIFIC CYSTEINE THIOLS OF ENDOTHELIAL CELL PROTEINS REGULATE ISCHEMIC BLOOD FLOW RECOVERY

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Introduction: Reactive oxygen species (ROS) during ischemia may affect the recovery of blood flow by inducing reversible cysteine (Cys) thiol oxidations (eg. S-glutathione (GSH) adducts) that initiate signaling. Metabolic stress (eg. in diabetes) causes greater increases in ROS and interferes with protein function. A mouse model of hindlimb ischemia (HLI) is being used to identify the key proteins and Cys involved.

Methods: HLI was induced by surgical femoral vascular excision. Blood flow was measured during 4 weeks with a laser Doppler flowmeter. Mouse microvascular endothelial cells (EC) were obtained from mouse heart on CD31 antibody coated beads and cultured. Angiogenic behavior (migration, network formation) was examined under control conditions and after metabolic stress exposure to high glucose and lipids. Protein GSH adducts were detected with a monoclonal antibody. Ca^{2+} was measured in EC with fura-2.

Results: HLI or exposure of EC to hypoxia rapidly increased protein GSH adducts. These could be decreased in vivo or in culture by overexpression of the specific protein thiol GSH transferase, glutaredoxin-1 (Glrx1). Glrx1 also prevented vascular EC growth factor (VEGF)-induced increases in EC Ca^{2+} and angiogenic behavior, and significantly impeded the recovery of HLI, indicating that some GSH protein adducts are essential for normal ischemic angiogenesis. Proteins with reactive Cys thiols which when adducted with GSH might explain altered blood flow include the sarco(endo)plasmic Ca^{2+} ATPase-2 (SERCA2), p21ras, and sirtuin-1. In the case of SERCA2, GSH adducts increased on the protein during ischemia or hypoxia. A SERCA2 C674S knockin mouse had impaired HLI recovery, and its EC showed impaired Ca^{2+} and angiogenic responses to VEGF and nitric oxide. In the case of p21ras, its angiogenic function depends upon plasma membrane Erk signaling, which is abrogated in EC when oxidants are increased by metabolic stress, in part because GSH adducts accumulate on terminal Cys-181,184 and prevent palmitoylation that normally accounts for p21ras plasma membrane localization. In the case of sirtuin-1, Cys GSH adducts inhibited its activity, and a multiple Cys to Serine mutant was superior to wild type protein in preserving EC angiogenic behavior during metabolic stress.

Conclusion: GSH adducts increase during ischemia on Cys thiols, affecting protein function and HLI. Some, like those on SERCA C674 appear to be essential for normal angiogenesis. Some, like those on p21ras and sirtuin-1 inhibit their angiogenic functions and come into play when metabolic stress further increases ROS. The orchestration of normal ischemic angiogenesis and its impairment by metabolic stress depends on GSH protein adducts, on the level of ROS, the reactivity of the specific Cys thiol, as well as the subcellular localization and the change in function of the specific protein.

ANGIOTENSIN CONVERTING ENZYME INHIBITION ENHANCES BRADYKININ RELAXATIONS THROUGH NITRIC OXIDE AND B1 RECEPTOR ACTIVATION IN BOVINE CORONARY ARTERIES

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Introduction: Bradykinin causes vascular relaxation through release of endothelial relaxing factors including prostacyclin, nitric oxide (NO) and epoxyeicosatrienoic acids (EETs). Bradykinin is metabolized by angiotensin converting enzyme (ACE) and ACE inhibition enhances bradykinin relaxations. Our goal was to characterize the role of bradykinin receptors and endothelial factors in ACE inhibitor-enhanced relaxations in bovine coronary arteries.

Methods and Results: In U46619 preconstricted arteries, the bradykinin 2 (B2) receptor agonist, bradykinin (10^-11 - 10^-8M) caused concentration-dependent relaxations (maximal relaxation = 122±9%), log EC_50 = -9.5±0.1). In the presence of the NO synthase inhibitor, N-nitro-L-arginine (L-NA, 3x10^-5M) relaxations were reduced by an inhibitor of EET synthesis, miconazole (maximal relaxation = 122±9%), log EC_50 = -9.5±0.1). In the presence of the NO synthase inhibitor, N-nitro-L-arginine (L-NA, 3x10^-5M) and the cyclooxygenase inhibitor, indomethacin (10^-5M), relaxations were reduced by an inhibitor of EET synthesis, miconazole (maximal relaxation = 122±9%), log EC_50 = -9.5±0.1). In the case of sirtuin-1, Cys GSH adducts inhibited its activity, and a multiple Cys to Serine mutant was superior to wild type protein in preserving EC angiogenic behavior during metabolic stress.

Conclusions: Our results demonstrate that ACE inhibitor-enhanced bradykinin relaxations of bovine coronary arteries occur through endothelial cell B1 receptor activation and NO.
**Phosphorylation on Thr495**

**AMP-ACTIVATED KINASE ALPHA1 SUBUNIT IN ENDOTHELIAL CELLS REGULATES VASCULAR REACTIVITY VIA eNOS PHOSPHORYLATION ON THR495**

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### Introduction

The AMP-activated protein kinase (AMPK) has frequently been reported to phosphorylate the endothelial nitric oxide synthase (eNOS) on Ser1177 and is therefore assumed to mediate a relaxing effect. However, previous studies failed to consistently demonstrate a major role for the AMPK on eNOS-dependent relaxation – largely because of the use of unspecific pharmacological AMPK activators and inhibitors. However, the AMPK can potentially phosphorylate the eNOS on a second inhibitory site i.e., Thr495 in the calmodulin binding domain to inhibit NO production. The aim of the present study was to determine the role of AMPKalpha1 and -alpha2 subunits in regulating NO-mediated vascular relaxation.

### Methods

Vascular reactivity was assessed in aortic rings and carotid artery segments from mice lacking the 2 subunits (AMPKalpha) or specifically in endothelial cells (AMPKalpha^DELTAEC) or the corresponding wild-type mice. Concentration response curves to phenylephrine as well as acetylcholine were generated in rings with and without intact endothelium, as well as in the absence and presence of the NOS inhibitor, N nitro-L-arginine methyl ester (L-NAME). In human umbilical vein endothelial cells, eNOS phosphorylation on Ser1177 and Thr495 were assessed after AMPK activation with thrombin (1 mmol/L, 5-60 minutes). Stretch-induced (isometric 15% or pulsatile 12%, 1 Hz) changes in eNOS phosphorylation were also assessed in cultured endothelial cells lacking AMPKalpha1.

### Results

The global deletion of neither the AMPKalpha1 nor alpha2 subunit affected the phenylephrine-induced contraction or the endothelium-dependent relaxation elicited by acetylcholine. The endothelial cell-specific deletion of the AMPKalpha1 subunit attenuated the phenylephrine-mediated contraction in an L-NAME and endothelium-dependent manner without significantly affecting acetylcholine-induced relaxation. These data indicated that basal NO production was increased in the absence of AMPKalpha1. In in vitro studies, activation of AMPK with thrombin did not alter the phosphorylation of eNOS on Ser1177 but increased phosphorylation on Thr495. The phosphorylation of eNOS on Ser1177 and Thr495 was comparable in cultured endothelial cells from wild type and AMPKalpha1 mice under basal conditions. However, there was a decrease in phosphorylation of eNOS on Thr495 in response to pulses in the cells lacking AMPKalpha1.

### Conclusion

The results of this study indicate that AMPKalpha1 targets the inhibitory phosphorylation Thr495 site in the calmodulin-binding domain of eNOS to attenuate basal NO production and phenylephrine-induced vascular contraction.
Endothelial Function

ROUX-EN-Y GASTRIC BYPASS IMMEDIATELY IMPROVES ENDOTHELIAL DYSFUNCTION AND HDL PROPERTIES BY A GLUCAGON LIKE PEPTIDE-1 MEDIATED BODY WEIGHT LOSS INDEPENDENTLY OF BODY WEIGHT LOSS


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Introduction: Roux-en-Y gastric bypass (RYGB) reduces weight and long-term cardiovascular (CV) risk in obese patients. The mechanisms underlying these CV protective effects of RYGB are yet unclear, but may be partly body weight independent. Glucagon like peptide-1 (GLP-1) increases after RYGB and activates endothelial-NO-synthase (eNOS). Here, we investigated whether GLP-1 plays a role in obesity-induced effects on endothelial and HDL function in rats after RYGB, prior to significant weight loss.

Methods: After 7 weeks on a high-fat high-cholesterol diet, male Wistar rats underwent RYGB or sham surgery. Sham rats were fed ad lib (AL) or body weight-matched (BWM) to RYGB. Thoracic aortic rings were collected 8 days post-surgery and suspended for isometric tension recording. Cumulative relaxation responses were performed to peptide GLP-1 (7–36)amide (10^-12 to 10^-6mol/L) after submaximal contraction with norepinephrine (10^-6mol/L), and after pre-incubation with the GLP-1 antagonist exendin (9-39) (10^-7 mol/L) or the eNOS-inhibitor L-NAME (10^-4mol/L). Western blot of aortic lysates using GLP-1 receptor and eNOS antibodies was performed to address the role of GLP-1 signaling in endothelial function. Arylesterase activity of HDL-associated paraoxonase (PON1) activity was measured by spectrophotometry using the substrates phenylacetate. HDL reverse cholesterol transport (RCT) capacity was measured ex vivo incubating J774 macrophages with apolipoprotein B-depleted rat serum. GLP-1 and bile acids plasma fasting levels were measured.

Results: On day 8 post-surgery, GLP-1-induced vasorelaxation was impaired in AL and BWM compared with RYGB rats (max relaxations: 17±3.1 % vs 15±2.8 vs 15±2.8, resp., n=6-8, p<0.05). Exendin (9-39) and L-NAME inhibited GLP-1-induced vasodilation, suggesting a mechanism requiring the known GLP-1 receptor and activation of eNOS. GLP-1 receptor protein expression was lower in aortic lysates from sham AL and BWM compared to RYGB (AL 0.44±0.1 vs BWM 0.49±0.1 vs RYGB 0.86±0.2 relative units, p<0.05); eNOS expression was also reduced (0.45±0.1 vs 0.34±0.1 vs 0.74±0.1). Plasma fasting levels of GLP-1 were higher after RYGB compared to sham AL and BWM (AL 0.8±0.1; BWM 2.0±0.9; RYGB 10±2.8 pg/ml, p<0.05). Plasma bile acids, which stimulate GLP-1 secretion from L cells in the lower intestine, were also increased in RYGB compared to both sham AL and BWM. PON1 activity increased only after RYGB compared to BWM, whereas it was impaired in AL. Moreover, HDL-RCT capacity improved only after RYGB compared to BWM and was lower in AL than in BWM rats.

Conclusion: Our study shows an immediate improvement of endothelial and HDL functions after RYGB likely mediated by GLP-1; all effects appeared to be independent from body weight loss.

PERIVASCULAR VISCERAL FAT CAUSES ENDOTHELIAL DYSFUNCTION OF MESENTERIC RESISTANCE ARTERIES IN A RAT MODEL OF METABOLIC SYNDROME (SHROB). ROLE OF PROSTAGLANDINS

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Visceral obesity is a cardinal symptom of metabolic syndrome. We recently observed in a rat model of this syndrome, the SHR obese (SHROB), that resistance arteries within the mesenteric visceral fat depot exhibit an important endothelial dysfunction. Our purpose is to determine if the fat surrounding these vessels accounts for this anomaly and if this is the case, whether it does so by altering NO or by the action of vasoactive prostaglandins released by perivascular adipocytes, namely: PGE2, PGJ2 and TXA2. Mesenteric resistance arteries (MRA) from SHROB and control rats (WKY) were mounted on wire myographs: a) together with a sphere of naturally occurring perivascular adipose tissue (with-PV AT group), or b) dissecting all the adventitial tissue (without-PV AT group). Endothelial function, tested by acetylcholine reactivity of SHROB arteries with PV AT, was markedly lower than that of WKY. With-PVAT arteries, especially the SHROB, showed lower responses than those without PVAT. NO synthase inhibition diminished the acetylcholine responses in every group except the with-PVAT SHROB. Blockade of cyclooxygenase II, PGJ2-IP, TXA2-TP, or TXA2 synthase increased to different extents the responses of the SHROB with-PVAT group. Immunoassay confirmed the release of PGE2, PGJ2 and TXA2 and PVAT of both strains revealed cyclooxygenase II activity and expression. We conclude: a) the presence of visceral PVAT causes endothelial NO dysfunction of resistance arteries in the SHROB; b) PVAT is a source of vasoactive prostaglandins in WKY and SHROB; and c) the pattern of PVAT release of vasoactive prostaglandins and vascular response to these partly underlies the endothelial dysfunction of SHROB arteries.
MECHANISMS UNDERLYING THE VASODILATORY EFFECTS OF CYSTAMINE IN SMALL MESENTERIC ARTERIES

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Introduction: The tissue transglutaminase (t-TG) inhibitor cystamine has been shown to be neuroprotective and to attenuate structural vascular alterations characteristic for essential hypertension. Vasodilatation may contribute to these effects. The present study hypothesized that cystamine evokes small vessel relaxation by blocking smooth muscle calcium entry and/or inhibition of Rho kinase.

Methods: Contraction studies, fura-2 measurements of intracellular Ca2+ ([Ca2+]i) and Western blot determination of phosphorylation of myosin phosphatase targeting subunit 1 (MYPT1) and myosin regulatory light chain (MLC2) were used to study the effects of cystamine in rat mesenteric small arteries.

Results: Cystamine (100 μM) rightward shifted and successively depressed concentration-response curves for phenylephrine, 5-hydroxytryptamine (5-HT) and and the thromboxane analogue, U46619, while 1 mM cystamine was required to suppress concentration-response curves for extracellular K+. In phenylephrine-contracted arteries, cystamine caused concentration-dependent relaxations and reduced [Ca2+]i, while in potassium-contracted arteries, cystamine induced less relaxation and without changing [Ca2+]i. In contrast to inhibitors of calcium-activated and ATP-sensitive K channels, the inhibitor of voltage-gated potassium KV7 channels, XE991 inhibited relaxations induced by low cystamine concentrations. Incubation with a suicide substrate for transglutaminase, cadaverine, inhibited relaxations induced by cystamine and by a selective inhibitor of transglutaminase 2. Cystamine relaxations were also inhibited by an inhibitor of phospholipase C. Phenylephrine increased myosin phosphatase targeting subunit 1 (MYPT1)-Thr855 and myosin light chain 2 phosphorylation. Cystamine and the Rho kinase inhibitor Y27632 (1 μM) reduced basal MYPT1-Thr855 phosphorylation, but only Y27632 reduced phenylhydrazine-induced MYPT1-Thr855 phosphorylation. MLC2 phosphorylation was unaltered in the presence of cystamine.

Conclusions: Our findings suggest that cystamine by inhibition of receptor-coupled transglutaminase leads to opening of KV7 channels and reduction of intracellular calcium as well as to force suppression which is followed by vasodilatation in rat small mesenteric. The vasodilator effect may contribute to the inhibitory effects of cystamine on the vascular remodelling characteristic for essential hypertension.

MATERNAL NUTRIENT RESTRICTION DURING PREGNANCY IMPAIRS AN ENDOTHELIUM-DEPENDENT HYPERPOLARIZING FACTOR-LIKE PATHWAY AND ENHANCES THE ROLE OF NITRIC OXIDE IN RELAXATION OF FETAL SHEEP CORONARY ARTERIES

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Introduction: Epidemiological studies suggest that low birth weight is linked to an increased incidence of cardiovascular disease in adulthood. The mechanisms underlying developmental programming are poorly understood but may be associated with adaptations by the fetus in response to changes in the maternal environment during pregnancy.

Hypothesis: Maternal nutrient restriction during pregnancy alters vasodilator responses in fetal coronary arteries.

Methods and Results: Pregnant ewes were fed a control (100% NRC) or nutrient restricted (60% NRC) diet from day 50-130 of gestation (term=145 days). In coronary arteries isolated from fetal lambs (130 days gestation), relaxations to bradykinin (10^-10 to 10^-7 M) were similar in tissues from control (pD_2= 8.06±0.3; E_max= 86±10% relaxation; n=6) and nutrient-restricted animals (pD_2= 8.22±0.3; E_max= 84±8% relaxation; n=6; P>0.05), and were unaffected by indomethacin (10^-5 M). In the presence of nitro-I-arginine (NLA; 3 x 10^-5 M), relaxation to bradykinin was fully suppressed and converted to a contractile response in rings from control animals. The NLA-resistant response to bradykinin in rings from control animals was nearly abolished in the presence of iberiotoxin (10^-7 M), a selective BK_Ca blocker, and by depolarization with K+ (40 mM). The selective K ATP blocker, glyburide (10^-6 M), had no effect. Quantitative RT-PCR identified the expression of BK_Ca alpha- and beta-subunits in fetal coronary arteries, which did not differ between control and nutrient-restricted animals. Whole-cell BK_Ca currents were nearly identical in coronary smooth muscle cells from control and nutrient-restricted animals (peak current density= 46±5 vs. 39±6 pA/pF; n=6). 14,15-EET (putative EDHF in coronary arteries) caused fetal coronary artery relaxation and BK_Ca activation that was unaffected by maternal nutrient restriction. Similar results were obtained with the BK_Ca-opener, BMS 191011 and NS1619.

Conclusion: Although there is no quantitative difference in bradykinin-induced relaxation of fetal coronary arteries from control and nutrient-restricted ewes, the underlying mechanisms differ. In control animals, bradykinin-induced relaxation is mediated primarily via a pathway independent of NO or prostacyclin and is dependent on activation of BK_Ca in coronary smooth muscle (i.e. EDHF-like). Maternal undernutrition during pregnancy results in loss of the EDHF-like pathway in fetal coronary arteries, an effect that is likely due to endothelial dysfunction (i.e. impaired synthesis and/or release of an EDHF-like mediator) rather than impaired BK_Ca activity in coronary smooth muscle cells. In fetal coronary arteries from nutrient-restricted animals, bradykinin-induced relaxation is completely dependent on the release of NO, which may represent an adaptive response to ensure adequate coronary perfusion.
**Oral Presentations**

**Endothelin-1 and Vascular Control**

1

NEGATIVE ALLOSTERIC MODULATION OF ENDOTHELIN ET\_A RECEPTOR FUNCTION IN RESISTANCE ARTERIES

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Inhibition of resistance artery responses to endothelins (ETs) might be useful in therapy-resistant hypertension and stroke. ETs cause long-lasting arterial contractions by tight binding to smooth muscle ET\_A receptors. Chemically diverse ET-receptor antagonists (ERA) inhibit binding of ET-1 to ET\_A and reduce sensitivity to ET\_A. We investigated negative allosteric modulation by ERA in isolated rat resistance arteries. 1 µM BQ123 (cyclic pentapeptide) reversibly relaxed contractile responses to 32 nM ET-1 to a different extent in arteries from different vascular beds (-0 to -80%) and reduced mesenteric artery (MA) sensitivity to ET-1 more avidly (pKB 7.6 ±0.4) than that to ET-2 (pKB 5.6±0.4). This agonist-dependence was less marked with 100 nM PD-156707 (butenolide; pKB 8.5 ±0.3 and 7.9 ±0.3) and not significant with 1 nM BMS-193884 (biphenyl sulphonamide; pKB 9.3±0.1 and 9.2±0.1). Effects of high concentrations of BMS-193884 on MA sensitivity to ET-1 and ET-2 were not concentration-dependent. In the presence of 10 nM BMS-193884, i) 1 µM BQ123 had no additional effect but ii) 100 nM PD-156707 resulted in a further significant reduction of MA sensitivity to ET-1. Binding i) a fluorophore to PD-156707 (useful for diagnosis) or ii) an angiotensin AT\_1-antagonistic moiety to BMS-193884 (dual antagonist PS-433540) impaired ET-antagonism by the pharmacophores. Thus, chemically distinct ERA act differently on resting and agonist-activated ET\_A receptors and display system- and agonist-dependence and saturability (pharmacological properties of allosteric modulation). Also, the observations suggest presence of several allosteric binding sites on ET\_A receptors, the structure-activity relationships of which can be studied.

2

LOW-GRADE INFLAMMATION PARTICIPATES ON THE ENHANCED VASOCONSTRICTION TO THE ENDOGENOUS ENDOTHELIN-1 IN SMALL VESSELS FROM OBESE PATIENTS

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**Introduction:** Obesity is characterized by vascular low-grade inflammation, with an augmented endogenous endothelin(ET)-\textsubscript{1}-mediated vasoconstriction (VC) and a blunted tonic nitric oxid(NO)-mediated relaxation (VD). We evaluated whether TNF-alpha, localized in the vascular wall and in perivascular adipocytes (PVA), contributes to the VC induced by endogenous ET-1 and whether this action is mediated by an effect on tonic NO release in small resistance arteries from obese patients (Ob) and controls (Ctrl).

**Method:** Sample of visceral fat were obtained in 14 Ob (BMI:42.1±3.7) and 14 Ctrl (BMI:25.4±3.6), matched for metabolic profile and blood pressure values, undergoing a surgical laparoscopic procedure. Small arteries were investigated on a pressurized micromyograph. Endogenous ET-1 was assessed by vascular response to BQ-123. TNF-alpha and NO were assessed by Infliximab (IFX) and L-NAME, respectively. Gene and protein expression of TNF-alpha, ET-1 and ETA receptors were determined by RT-PCR (gene/reference) and IHC (HU) on arterial wall and in PVA.

**Results:** In Ctrl, L-NAME-induced VC (15.5±0.6%) was not affected by IFX (15.1±0.4%). In contrast, Ob showed a blunted VC to L-NAME (6.0±0.7%; P<0.01 vs Ctrl) which was potentiated (P<0.01) by IFX (12.5±0.8%). In Ob, the VD to BQ-123 (47.0±1.5%) was attenuated (P<0.01) by IFX (29.1±2.4%) and not affected by L-NAME (43.3±0.6%). During IFX co-infusion, L-NAME further reduced the VD to BQ-123 (19.4±3.0%; P<0.01 vs BQ-123+IFX). In Ctrl, VD to BQ-123 was blunted (26.3±1.3%; P<0.01 vs Ob), not affected by IFX (24.1±0.6%) and significantly reduced by L-NAME (12.3±1.1%; P<0.05 vs BQ-123). Ob showed a significant overexpression of TNF-alpha respect to Ctrl, either at the level of arterial wall (24.9±19.6 vs 2.8±2.5 AU, P<0.001) or in PVA (2.9±1.8 vs 1.2±0.7 gene/reference, P=0.005). These results were paralleled by a higher arterial expression of ET-1 (45.8±10.3 vs 24.3±15.0 AU, P=0.001) and ETA receptors (69.4±6.0 vs 9.6±2.8 AU, P<0.001) in Ob vs Ctrl.

**Conclusion:** Small vessels of Ob show an enhanced ET\_\textsubscript{1}-mediated VC and a blunted NO-mediated VD. An excess of vascular and perivascular TNF-alpha, coupled with an increased expression of ET\_\textsubscript{1} and ETA in the vasculature of Ob, contributes to the enhanced ET\_\textsubscript{1}-mediated VC tone partly by an impairment of tonic NO release.
Background: Transient left ventricular apical ballooning syndrome or Takotsubo cardiomyopathy is an acute cardiac syndrome mimicking ST-segment elevation myocardial infarction. This syndrome is typically observed in patients experiencing sudden physical or emotional stress. The precise aetiology and pathophysiology of this syndrome as well as its prognosis remain unclear. Aim of this study was to evaluate sympathetic nervous activity and vascular function and structure, in patients with Takotsubo syndrome and matched controls.

Methods: 22 patients with Takotsubo-syndrome and 21 controls, matched for age, cardiovascular risk factors and medications were included in this prospective observational study. Sympathetic activation was assessed via microneuromography, flow-mediated vasodilation of the brachial artery was used as a measure of endothelial function at baseline and during stress tests, arterial stiffness was evaluate by measurement of pulse wave velocity, carotid atherosclerosis was assessed as intima media thickness and total plaque area and quality of life by EQ5 questionnaire.

Results: Compared to controls, patients with Takotsubo syndrome were characterized by a significantly reduced endothelial function (FMD 3.0±1.7% vs. 5.0±1.7%; p<0.005), and an increased sympathetic nervous activity (MSA 49.7±27.5 vs. 28.7±15.1 Burst/100HB). Cardiovascular risk factors (age, weight, glucose, lipid profile, physical activity) as well pharmacological therapies, pulse wave velocity, intima-media-thickness and total plaque area as well as quality of life were similar in both groups.

Conclusion: Our findings highlight the potentially important role of sympathetic activation and endothelial dysfunction in patients with the Takotsubo syndrome.

ENDOTHELIA GLYCOALYX ACCESSIBILITY DETERMINES MICROVASCULAR PERFUSION AND METABOLIC BALANCE IN OBESITY

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INTRODUCTION: One of the earliest changes in the development of cardiovascular disease is the degradation and modification of the endothelial glycocalyx (endothelial surface layer). The endothelial glycocalyx is both an important biological modifier of interactions between flowing blood and the vessel wall and a determinant of organ perfusion. In a cohort of overweight/obese participants, we have utilized non-invasive sidestream darkfield (SDF) imaging to investigate the hypothesis that changes in endothelial glycocalyx accessibility by passing red blood cells (RBC) are associated with changes in microvascular perfusion and overall metabolism.

METHOD: The Netherlands Epidemiology of Obesity (NEO) study is a population-based prospective cohort study of 6,673 middle-aged individuals, with oversampling of overweight and obese individuals, to study pathways that lead to obesity-related diseases. Within this cohort, we have imaged the sublingual microvasculature of 919 participants using SDF imaging together with a recently developed automated acquisition and analysis software. The software selects and measures microvessels with a maximum RBC column width of 25 micrometers. Within these microvessels, the RBC filling percentage (the fractional longitudinal RBC density, derived from the spatio-temporal presence of RBC) and microvascular perfusion efficiency (RBC filling percentage × functional capillary density percentage, being a relative measure of well perfused microvascular fraction) were calculated. In addition, as a measure of the glycocalyx barrier properties, the perfused boundary region (PBR) was calculated. Since microvascular perfusion affects muscle metabolism, we validated this relation using the resting indirect calorimetric respiratory quotient (RQ, being VCO₂/V VO₂).

RESULTS: Within the study group, a wide range of variability in PBR measurements, with a mean PBR of 2.12 µm, (95%CI [1.71 – 2.54]), was observed. Since follow up of the cohort is ongoing we cannot relate this variability to development of CV disease at this stage. However, PBR showed a very strong inverse correlation with both the RBC filling percentage (73.3%, 95%CI [65.0 – 81.1]) and microvascular perfusion efficiency (54.9%, 95%CI [40.0 – 68.0]); Spearman’s rho = -0.683 and -0.566, respectively at a 2-tailed 0.01 significance level). Changes in PBR and RBC filling both correlated with changes in the resting metabolic state, reflected by the respiratory quotient (mean 0.84, 95%CI [0.76 – 0.94] (N = 206); Spearman’s rho = -0.141 and 0.141 respectively, both at a 2-tailed 0.05 significance level).

CONCLUSION: SDF imaging of the mucosal microvasculature in obese subjects showed a wide range of variability in RBC accessibility to the glycocalyx which correlated inversely with microvascular perfusion and resting metabolic state. This means that with a thick (“healthy”) glycocalyx (reflected by a low PBR) there is preferential perfusion of the microvascular bed and metabolic balance. In contrast, a thin (“risk”) glycocalyx (reflected by a high PBR) is associated with a diffuse, less efficient perfusion and a tendency for carbohydrate oxidation and fat accumulation.
3

EFFECUTS OF MENOPAUSAL HORMONE TREATMENTS ON PLATELET ATP SECRETION AND VASOCACTIVE PROSTANOIDS IN RECENTLY MENOPAUSAL WOMEN.

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Introduction: Menopausal hormone treatment (MHT) may slow progression of cardiovascular disease but increase risk of thrombosis. Transdermal MHT may carry less risk of thrombosis than oral MHT. Activated platelets secrete vasoactive molecules as well as provide a surface for generation of thrombin. However, effects of oral compared to transdermal MHT on platelet secretion have not been investigated. Two prostanoids have opposing effects on platelets: prostacyclin (PGI2), a vasodilator, inhibits platelet aggregation and secretion; whereas, thromboxane (TXA2), a vasoconstrictor, induces platelet aggregation and secretion. Thus, their balance is critical to maintain vascular homeostasis. This study evaluated platelet dense granule ATP secretion in relationship to PGI2, and TXA2 among women enrolled in the double blinded, placebo controlled randomized Kronos Early Estrogen Prevention Study (KEEPS).

Methods: Women (average age 52.6 years, n=118) without cardiovascular disease and within three years of menopause were randomized to: 1) Placebo (pills and patch, PL), 2) Transdermal 17beta-estradiol (50 microg/week, tE2), or 3) Oral conjugated equine estrogen (0.45 mg/day, oCEE) with oral progesterone, 200 mg/first 12 days of each month for 48 months. Platelet-rich plasma was prepared from anti-coagulated blood and quantified by Coulter counter. Platelet ATP secretion in response to thrombin receptor agonist peptide (TRAP, 10 microM) was determined in the presence and absence of prostaglandin E1 (PGE1, ~500nM) which, like prostacyclin raises platelet cAMP and inhibits platelet activation. Washed platelets were placed in lysis buffer (% Triton X-100, 10 nM TRIS, pH 7.4), passed through a 26-gauge needle, then sonicated and pellets by centrifugation. PGI2 and TXA2 in lysates were evaluated by quantification of their stable metabolites TXB2 and 6-K-PGF1alpha using ELISA.

Results: After 48 months of treatment, the platelet count (range 234-249 x 10^3 platelets/microL), maximal ATP secretion in response to TRAP (range 20.3-35.3 atoamoles/platelet) and inhibition of ATP secretion after incubation with PGE1 (range 8-30%) were similar among the three groups. However, the ratio of 6-K-PGF1alpha to TXB2 trended higher in the oCEE group compared to tE2 and PL groups: 2.76 (n=13), 1.52 (n=20), 1.20 (n=21), respectively. Inhibition of platelet secretion by PGE1 positively correlated with TXB2 only in the oCEE group (r = 0.74, p=0.007).

Conclusion: Neither oCEE or tE2 significantly altered platelet number or maximal ATP secretion. However, oCEE increased both the ratio of 6-K-PGF1alpha to TXB2 and basal platelet cAMP which may render the platelet less sensitive to threshold stimuli for secretion. The known risk of thrombosis with oral MHT may not result from increased platelet secretory functions.

4

THE REGULATION OF BONE BLOOD FLOW AT REST AND DURING EXERCISE IN HUMANS

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Abstract

The beneficial changes in bone mineral content and structure induced by physical activity are likely made possible by increased blood flow that supplies bone with nutrients and oxygen in accordance with its mechanical and metabolic needs. However, the mechanisms that regulate bone blood flow in humans are largely unknown, although we have recently shown that human bone shows circulatory and metabolic activity in response to exercise and other physiological perturbations. Animal studies suggest that nitric oxide could be one of the main mediators of bone blood flow. Therefore, in the present study we investigated the effect of inhibition of nitric oxide synthase (NOS) on femoral bone blood flow by positron emission tomography in eight young healthy men at rest and during one leg dynamic exercise, with and without combined blockade with prostaglandins. In the second protocol, we also determined in a group of six separate young healthy men whether adenosine plays a role mediating bone hyperaemia during exercise (adenosine receptor inhibition by aminophylline). Inhibitions of the possible blood flow mediators were obtained by administration of blockers into the femoral artery. Resting bone blood flow was 1.1 ± 0.4 ml/100g/min and increased by almost 6-fold in response to exercise (6.3 ± 1.5 ml/100g/min). Inhibition of the formation of nitric oxide reduced bone blood flow at rest to 0.7 ± 0.3 ml/100g/min (p=0.036), but did not affect blood flow significantly during exercise 5.5 ± 1.4 ml/100g/min (p=0.25). On the other hand, while the addition of prostaglandin inhibition did not cause any further reduction of blood flow at rest (0.6 ± 0.2 ml/100g/min), as induced by NOS inhibition alone, the combined blockade of NOS and prostaglandins reduced bone blood flow during exercise by ~21%, to 5.0 ± 1.8 ml/100g/min (p=0.014). Finally, the inhibition of adenosine receptors during exercise reduced femoral blood flow from control flow of 5.5 ± 1.9 ml/100g/min to 4.6 ± 1.2 ml/100g/min (p=0.045). In conclusion, our results support the view based on animal studies that nitric oxide plays an important role in controlling bone blood flow at rest in humans as well, and together with prostaglandins controls blood flow during exercise. Further, also adenosine appears to be an important regulator of bone blood flow during exercise.
INVOLVEMENT OF CYTOCHROME EPOXYGENASE METABOLITES IN CUTANEOUS POSTOCCLUSIVE HYPEREMIA IN HUMANS

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Objective: Several mediators contribute to postocclusive reactive hyperemia (PORH) of the skin including sensory nerves and endothelium derived hyperpolarizing factors (EDHF). The main objective of our study was to investigate the specific contribution of epoxyeicosatrienoic acids in human skin PORH.

Methods: Eight healthy volunteers were enrolled in two placebo controlled experiments. In the first experiment we studied the separate and combined effects of 6.5 mM fluconazole, infused through microdialysis fibers, and lidocaïne/prilocaine cream on skin PORH following 5 min arterial occlusion. In the second experiment we studied the separate and combined effects of 6.5 mM fluconazole and 10 mM L-NMMA. Skin blood flux was recorded using two-dimensional Laser Speckle Contrast Imaging. Maximal CVC was obtained following 29 mM sodium nitroprusside perfusion.

Results: The PORH peak at the placebo site averaged 66 +/- 11 %CVCmax. Compared to the placebo site, the peak was significantly lower at the fluconazole (47 +/- 10 %CVCmax; P < 0.001); lidocaïne (29 +/- 10 %CVCmax; P< 0.001); and fluconazole + lidocaïne (30 +/- 10 %CVCmax; P < 0.001) sites (figure 1). The effect of fluconazole on the area under the curve was more pronounced. In the second experiment, the PORH peak was significantly lower at the fluconazole site, but not at the L-NMMA or combination site, compared to the placebo site.

Conclusions: In addition to sensory nerves cytochrome epoxygenase metabolites, putatively epoxyeicosatrienoic acids, play a major role in healthy skin PORH, their role being more important in the time course rather than the peak. ClinicalTrials.gov Identifier: NCT01290198

Figure 1: Representative Laser Speckle Contrast Imaging image over the 4 microdialysis fibers during the peak of postocclusive reactive hyperemia (region of interest 1 to) where the effect of fluconazole, lidocaïne/prilocaine and the combination can be observed.
Nitric Oxide and Soluble Guanylyl Cyclase

ENDOTHELIAL-SPECIFIC DELETION OF GCH1 REVEALS CELL-AUTONOMOUS REQUIREMENTS FOR ENDOTHELIAL TETRAHYDROBIOPTERIN IN REGULATION OF NITRIC OXIDE vs. HYDROGEN PEROXIDE-MEDIATED VASODILATATION AND BLOOD PRESSURE

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Nitric oxide (NO), produced by endothelial NO synthase (eNOS) is a key regulator of vascular tone and blood pressure. Tetrahydrobiopterin (BH4) is an essential cofactor for endothelial NOS. Increasing BH4 levels (e.g. by pharmacologic supplementation or transgene overexpression) can improve eNOS function and reduce superoxide anion production from eNOS uncoupling in disease states, but the physiological requirement for endothelial cell BH4 in normal vascular function remains unknown. In order to investigate the cell-specific requirements for BH4 in vivo, we generated mice homozygous for a floxed GCH1 (GCH1\textsuperscript{flo/flo}) allele (encoding GTPCH I, the rate-limiting enzyme in BH4 biosynthesis). Global deletion of GCH1 was embryonic lethal by day e12.5, but crossing with Tie2-cre transgenic mice produced healthy GCH1\textsuperscript{flo/flo}/Tie2-cre mice, where GCH1 is knocked out in specifically in endothelial cells. GCH1 expression, GTPCH protein and BH4 were selectively absent in GCH1\textsuperscript{flo/flo}/Tie2-cre endothelial cells, demonstrating that endothelial cell BH4 is not rescued by circulating BH4 in plasma. In aortas, eNOS protein was increased in GCH1\textsuperscript{flo/flo}/Tie2-cre mice and superoxide production, measured using dihydroethidium fluorescence, was 4-fold greater in GCH1\textsuperscript{flo/flo}/Tie2-cre mice compared to wild-type littermates, and was abolished by the NOS inhibitor L-NAME. Vasomotor studies demonstrated that GCH1\textsuperscript{flo/flo}/Tie2-cre aortas had enhanced vasoconstriction to phenylephrine (Emax, 97.8±7.3 vs. 118±5.6 %; P<0.05) that normalised in the presence of L-NAME. In small mesenteric resistance vessels from GCH1\textsuperscript{flo/flo}/Tie2-cre mice, constriction to U46619 was increased and relaxation to the PAR2 agonist SLIGRL was diminished. Endothelium-dependent vasodilatations in response to acetylcholine were impaired in vessels from GCH1\textsuperscript{flo/flo}/Tie2-cre vessels, whereas PEG-catalase had no effect in wild-type vessels. Ex vivo supplementation with the BH4 precursor sepiapterin restored normal endothelial function and abolished eNOS-derived H2O2 production in GCH1\textsuperscript{flo/flo}/Tie2-cre aortas. Further studies with catalase, ODQ and inhibitors of K-channels revealed that the effects of eNOS-derived H2O2 in GCH1\textsuperscript{flo/flo}/Tie2-cre are mediated by soluble guanylate cyclase. Blood pressure measurement using tail-cuff plethysmography demonstrated higher systolic blood pressure (114±1.1 in WT vs. 113±1.8 in GCH1\textsuperscript{flo/flo}/Tie2-cre mice) that normalised when L-NAME was given in drinking water (114±1.1 in WT vs. 113±1.8 in GCH1\textsuperscript{flo/flo}/Tie2-cre). These findings, in a new model of cell-specific deletion of GCH1, demonstrate that endothelial cell BH4 plays a pivotal, cell-autonomous role in maintaining normal vascular function by determining the formation of alternative eNOS-derived vasodilators, NO vs. H2O2. The regulation of NO vs. H2O2 production by endothelial cell BH4 may have important implications for vascular dysfunction and hemodynamic regulation in cardiovascular disease states.

NEW INSIGHTS INTO BIOACTIVATION AND EFFECTS OF ORGANIC NITRATES, NITRATE TOLERANCE AND CROSS-TOLERANCE: A MOLECULAR APPROACH

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The hemodynamic and antiischemic effects of nitroglycerin (GTN) are lost upon chronic administration due to the rapid development of nitrate tolerance. The mechanism of this phenomenon has puzzled several generations of scientists, but recent findings have lead to novel hypotheses. The formation of reactive oxygen and nitrogen species (RONS) in the mitochondria and the subsequent inhibition of the nitrate-bioactivating enzyme mitochondrial aldehyde dehydrogenase (ALDH-2) appear to play a central role, at least for GTN that is bioactivated by ALDH-2. Importantly, these findings provide the opportunity to reconcile the two "traditional" hypotheses of nitrate tolerance, i.e. the one postulating a decreased bioactivation and the concurrent one suggesting a role of oxidative stress. Nitrate-induced dysfunction (uncoupling) of endothelial nitric oxide synthase (eNOS) seems to represent a major oxidative side effect of several organic nitrates in clinical use. For those organic nitrates that are bioactivated by ALDH-2 (e.g. GTN and pentaerithrityl tetranitrate [PETN]), the redox-regulation of ALDH-2 (Fig. 1) may represent another side effect of nitrate therapy which is associated with reactive oxygen and nitrogen species formation. Further, recent animal and human experimental studies suggest that the organic nitrates are not a homogeneous group but demonstrate a broad diversity with regard to induction of vascular dysfunction, oxidative stress and other side-effects. In the past, attempts to avoid nitrate-induced side-effects have focused on administration schedules that would allow a "nitrate-free interval"; in the future, the role of cotherapies with antioxidant compounds and of activation of endogeneous protective pathways such as the heme oxygenase 1 (HO-1) by PETN will need to be explored. On the other hand, the development of new nitrates, e.g. tolerance-free aminooalkyl nitrates or combination of nitrate-groups with established cardiovascular drugs like ACE inhibitors or AT1-receptor blockers (hybride molecules) may be of great clinical interest.

Fig. 1. Redox-regulation of mitochondrial aldehyde dehydrogenase (ALDH-2) expression and activity.
Oral Presentations

Nitric Oxide and Soluble Guanylyl Cyclase

SOLUBLE GUANYLYL CYCLASE-DERIVED INOSINE 3':5'-CYCLIC MONOPHOSPHATE AS A MEDIATOR OF HYPOXIC CONSTRCTION OF PORCINE CORONARY ARTERY

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Introduction: Hypoxia has been found to cause vasoconstriction in a manner depending on endogenous or exogenous nitric oxide (NO) and sensitive to inhibition of soluble guanylyl cyclase (sGC), but the underlying mechanism remains unclear. The present study was to determine whether or not inosine 3':5'-cyclic monophosphate (cIMP), which can be converted from inosine 5'-triphosphate (ITP) by sGC, may act as a mediator of hypoxic vasoconstriction.

Method: Experiments were performed on isolated porcine coronary artery (outside diameter: ~5mm) with and without endothelium. Isometric tension of vessel rings was measured in organ chamber with a gas mixture containing 95% O2-5% CO2 for normoxia and 95% N2-5% CO2 for hypoxia. The phosphorylations of the regulatory subunit of myosin light chain phosphatase (MYPT1) at Thr-853 and myosin light chain (MLC) at Ser-19 were analyzed by Western blotting. The intracellular contents of cGMP and cIMP of arteries treated with IBMX were measured by HPLC-MS.

Results: In porcine coronary artery contracted with U46619 (3x10^-8 M - 3x10^-7M) and high potassium (30 mM - 60 mM) hypoxia caused a further contraction, which was abolished by endothelium removal, inhibition of eNOS with nitro-L-arginine, and inhibition of soluble guanylyl cyclase (sGC) with ODQ. The hypoxic effect occurred in artery without endothelium treated with exogenous NO donor (DETANONOate) or ITP in a manner sensitive to sGC inhibition. Hypoxia also caused contraction in artery denuded of endothelium treated with exogenous cIMP but not cGMP nor 8-Br-cGMP. Hypoxia increased phosphorylations of MYPT1 and MLC in arteries with but not without endothelium. Hypoxia also increased phosphorylation of MYPT1 and MLC in arteries denuded of endothelium treated with cIMP. HPLC-MS revealed a moderated decrease in cGMP level coincident with a marked increase in cIMP level in intact arteries exposed to hypoxia as compared with those exposed to normoxia. The effects were abolished by ODQ.

Conclusion: The present study demonstrates that hypoxia augmented contraction of porcine coronary arteries in a fashion depending on NO and sGC. The hypoxic effect may result at least in part from an increased conversion of cIMP by sGC under hypoxia and followed by increased phosphorylation of MYPT1 and MLC.
1 ENDOTHELIAL OVEREXPRESSION OF LOX-1 DECREASES ARETERIAL THROMBOSIS AND TF EXPRESSION IN VIVO: ROLE OF SIRT1 AND NFkB.

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Background-The hallmark of the initiation of atherosclerotic lesion is foam cell formation, and oxidized LDL (OxLDL) is believed to play a key role in the initiation of the atherosclerotic process. OxLDL is internalized by several receptors, except for SR-A/I, SR-BI, CD36, and CD68. OxLDL is also internalized by endothelial cells, but this uptake depends on receptors other than the classic scavenger receptors. In 1997, a lectin-like oxidized LDL receptor-1 (LOX-1, OLR1) was identified in bovine aortic endothelial cells. LOX-1 is a type II membrane glycoprotein with an apparent molecular weight of 50 kDa. It has a C-terminal extracellular C-type lectin-like domain. This lectin-like domain is essential for binding to OxLDL. Binding of OxLDL to LOX-1 induces several cellular events in endothelial cells, such as activation of transcription factor NF-kB, upregulation of MCP-1, and reduction in intracellular NO, which may trigger the onset of cardiovascular events or accelerate the development of atherosclerosis.

Methods and Results- We generated endothelial-specific LOX-1 transgenic mice using the Tie2 promoter (LOX-1TG). 12-week-old male LOX-1TG and wild-type (WT) mice were applied for carotid artery thrombosis model. LOX-1TG mice developed carotid artery thrombosis within a mean occlusion time of 36.96±4.83 min, while WT control mice occluded within a mean time period of 22.75±3.87 min (n=10, P < 0.05). Initial blood flow in carotid artery did not differ between both groups of mice. Decreased occlusion time in LOX-1TG mice was further associated with decreased tissue factor expression and surface activity as shown by RT PCR and ELISA. Furthermore, LOX-1TG mice showed increased mRNA expression of deacetylase SIRT1 in carotid artery, pointing out that SIRT1 may be involved in the observed downregulation of tissue factor through its known target transcription factor NF-kB.

Conclusions- Thus, our data suggest that LOX-1 plays a protective role in the arterial thrombosis and that SIRT1 may be involved. Therefore, both LOX-1 and SIRT1 may represent novel therapeutic targets for preventing arterial thrombosis.

2 THE ROLES OF SMOOTH MUSCLE NOX4 IN ATHEROSCLEROSIS AND RESTENOSIS

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Introduction: Atherosclerosis and stenosis are the leading causes of morbidity and mortality in developed countries. Elevated levels of reactive oxygen species (ROS) in the vascular wall play a key role in the development of atherosclerosis and stenosis. Nox4 based NADPH oxidases are the major ROS generating enzymes in the vasculature. Our previous study indicates that upregulation of Nox4 in smooth muscle cells (SMCs) accounts for the increased restenosis in pre-diabetic obese Zucker rats, indicating that SMC Nox4 may play an important role in restenosis. Different Nox deficient mice on ApoE-/ - background confirm the roles for p47phox, Nox2 and Nox1 in atherosclerosis. Surprisingly, Nox4 is the most abundant NADPH oxidase component in the vasculature, but its roles in atherosclerosis remain unclear.

Methods: Mouse lines to overexpress human Nox4 dominant negative form P437H (Nox4DN) in smooth muscle driven by the SM22-alpha promoter were characterized in a FVB/N background. The probe at 437 is in the NADPH binding domain, and its substitution to histidine abolishes ROS production. Furthermore, this mutation functions as a dominant negative against endogenous Nox4 by competing for the required interaction with p22phox. Non-transgenic littermate mice (NTg) were used as controls. These Nox4DN mice were backcrossed into FVB/N ApoE deficient background for atherosclerotic lesion analysis.

Results: In FVB/N background, Nox4DN significantly decreased ROS production, serum induced proliferation and migration, and macrophage adhesion in aortic SMCs compared with NTg. Nox4DN modestly but significantly decreased blood pressure. In a wire injury induced endothelial denudation model, Nox4DN significantly decreased neointima formation. In FVB/N ApoE deficient background, Nox4DN significantly decreased western diet induced aortic root lesions and aortic pulse wave velocity. In a partial left carotid artery ligation model to acutely induce disturbed flow, the mRNA level of Nox4 was significantly increased in smooth muscle. In this model, Nox4DN significantly decreased the neointima area and oil red O positive area, indicating decreased lipid accumulation. Gene expression assays indicated that soluble epoxide hydrolase 2 (sEH) and thrombospondin 1 (TSP1) were significantly downregulated in Nox4DN, which were furthered confirmed in protein levels. In NTg SMCs, downregulation of either sEH or TSP1 decreased cell proliferation and migration, but only downregulation of sEH suppressed inflammation and macrophage adhesion. Downregulation of TSP1 had no effect on sEH, while knockdown of sEH decreased TSP1, indicating that sEH can directly regulate TSP1.

Conclusion: Downregulation of smooth muscle Nox4 inhibits restenosis and atherosclerosis by suppressing sEH, which inhibits SMC proliferation, migration and inflammation.
DELETION OF HEPATIC ERK2 DECREASED THE SERCA2 EXPRESSIONS, WHICH CAN ACCOUNT FOR VASCULAR OXIDATIVE STRESS AND ENDOTHELIAL DYSFUNCTION IN METABOLIC STRESS.

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Introduction: Previously we identified that the dysfunction of vascular sarco/endoplasmic reticulum (ER) Ca2+-ATPase 2 (SERCA2) was a target for atherosclerosis and diabetes. In recent reports, the decreased hepatic SERCA2 function can cause hepatosteatosis (HST) in metabolic stress. Although HST is associated with vascular diseases in obese-related diseases, the role of HST in endothelial dysfunction was not clarified. We have created liver-specific ERK2 knockout mice (LE2KO) and fed them with a high-fat/high-sucrose diet (HFHSD) for 20 weeks, which revealed the marked deterioration of HST. The aim of study is to test the involvement of hepatic SERCA2 with ER stress in endothelial dysfunction associated with the imbalance of NO/oxidative stress.

Method: A hepatoma cell-line, Huh-7 cells were exposed to palmitate (250 mM, 24hr) with or without a MEK inhibitor PD98059 and tested the SERCA expression and ER stress. LE2KO was fed with HFHSD for 20 weeks to induce HST without changing blood pressure, serum LDL-C/HDL-C/TG levels, and the expressions of SERCA2 and the ER stress were tested. Serum glucose, free fatty acids (FFA), insulin, peroxide products (dROM), adiponectin levels were measured. Vascular oxidative stress was assessed with dehydroethidium staining and amplex red methods. Acetylcholine (ACh)- and sodium nitropurruside (SNP)-induced relaxation were measured with aortic rings from LE2KO and Control mice with the normal chow or HFHSD.

Results: An inhibition of ERK with PD98059 decreased the SERCA2 expression, and increased the phosphorylation of IRE and the expression of CHOP and BIP in Huh-7 only exposed with palmitate. HFHSD-fed LE2KO also decreased the hepatic SERCA2 expression, and increased the phosphorylation of IRE, PERK, eIF-2 and JNK, and the expression of CHOP and BIP. HST with an accumulation of TG in liver caused metabolic remodeling represented by increases in serum glucose, insulin, FFA, and dROM levels in LE2KO with HFHSD. The serum adiponectin level was decreased in LE2KO with HFHSD. In aorta from LE2KO with HFHSD, DHE staining and H₂O₂ production assessed with amplex red were elevated. The phosphorylation of eNOS at Ser1177 was decreased in them. ACh-induced relaxation was markedly impaired and SNP-induced relaxation was preserved in aorta from LE2KO with HFHSD.

Conclusion: The deletion of hepatic ERK2 decreased the hepatic SERCA2 expression with high fat, which caused hepatic ER stress and metabolic remodeling in vivo. Multiple factors, including the elevation of serum insulin, glucose, and FFA levels, the induction of systemic oxidative stress and the reduction of serum adiponectin levels, were observed in HST of the model. The vascular oxidative stress and the endothelial dysfunction with the impairment of eNOS phosphorylation were observed. These findings demonstrated that the hepatic ERK2/SERCA2 cascade could be a novel pathway to protect from endothelial dysfunction in metabolic stress of obese-related diseases.
ENDOTHELIAL ANGIOTENSIN CONVERTING ENZYME (ACE) LEVELS DECREASE IN RESPONSE TO ACTIVATION OF THE AMP-ACTIVATED PROTEIN KINASE (AMPK) VIA P53 AND MICRO-RNA(miR)-143/145

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Introduction: ACE is a key enzyme in the renin-angiotensin-system and plays a major role in blood pressure regulation. Although high ACE levels are a risk factor for cardiovascular disease development, little is known about the mechanisms regulating endothelial ACE expression. Here, we determined the role of AMPK and the miR-143/145-cluster, since they regulate ACE expression in other cell types and are activated/upregulated in response to shear stress.

Method and Results: Application of shear stress time-dependently decreased ACE expression in cultured human endothelial cells, an effect prevented by downregulating (siRNA) the catalytic AMPKα2 (but not alpha1) subunit. The AMPK activators, AICAR and metformin also decreased ACE expression in cultured endothelial cells and endothelial cells from AMPKα2 knockout mice. In miR-143/145-deficient LacZ reporter gene mice, ACE was identified as target of the miR-143/145 cluster in vascular smooth muscle cells. We confirmed the suppressive effect of miR-143/145 on ACE expression in isolated endothelial cells from these mice as well as in human endothelial cells by overexpressing miR-143/145. Moreover, AMPKα2 deletion (in vitro and in vivo) decreased miR-143/145 levels. Because shear stress increased levels of premature and mature miR-143/145, without affecting the primary transcript or miR-143/145 promoter activity, it seems that AMPKα2 regulates miR expression via a post-transcriptional rather than transcriptional mechanism. Since p53 is an AMPK target, that can affect miRs at post-transcriptional levels and since shear-stress elicited the AMPKα2-mediated phosphorylation of p53 (Ser15), we focused on validating AMPK-p53 effect on miR processing. Indeed, suppression of p53 (siRNA) decreased miR-143/145 levels, increased endothelial ACE expression and prevented its shear stress-induced downregulation. Streptozotocin-induced diabetes in mice was studied as a pathophysiological model of altered AMPK activity. Diabetes increased tissue phosphorylation of the AMPK substrates, p53 and acetyl-coenzyme A carboxylase, changes that correlated with increased miR-143/145 levels and decreased ACE-expression.

Conclusion: Activation of AMPKα2 suppresses endothelial ACE expression via p53 activation and post-transcriptional upregulation of miR-143/145. Dysregulation of AMPK or p53 plays a major role in the pathology of several diseases (e.g. diabetes, cancer) and their effect on miR-143/145 processing and thus ACE levels might mediate disease-associated cardiovascular disorders.
THE ROLE OF miR-483-3P IN ENDOTHELIAL HOMEOSTASIS AND RESPONSE TO VASCULAR INJURY

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Aim of the study: We have investigated the role of the micro-RNA miR-483-3p - which has previously only been known to be involved in modulating hepatocarcinoma growth - for the maintenance of endothelial homeostasis and for the response to acute vascular injury.

Methods: Expression of the miR-483-3p was assessed by RT-qPCR in human aortic endothelial cells (HAEC) and in myeloid early outgrowth cells (EOC) obtained from healthy volunteers (H), as well as from patients with stable coronary artery disease (CAD) with or without additional type 2 diabetes mellitus (T2D). HAEC or EOC were transfected with precursor of miR-483-3p (mi483) or Power Inhibitor of miR-483-3p (anti483), as compared to scrambled oligonucleotide control (scr). After 24h, survival, proliferation and contribution of the transfected cell to in vitro and in vivo re-endothelialization were assessed.

Results: We observed 1.7-fold to 3.0-fold higher expression levels of miR-483-3p in EOCs from CAD-T2D patients as compared to H controls and non-diabetic CAD patients (respectively; P<0.05). Transfection of HAEC with miR483 induced apoptosis in HAEC (mi483: 13.6±3.1% vs. scr: 4.2±1.7%; P=0.004) and impaired HAEC in vitro re-endothelialization capacity (mi483: -0.5±3.2% vs. scr: 7.9±1.0%; p=0.03). Transfection of EOC from H volunteers with miR483 reduced their capacity to support re-endothelialization in vitro and in a mouse model of acute vascular injury (mi483: 33.5±3.2% vs. scr: 24.8±2.3%; P<0.05). Vice versa, transfection of EOC obtained from CAD-T2D patients with anti483 enhanced their capacity to support in vivo re-endothelialization (anti483: 31.2±3.1% vs. scr: 21.6±2.6%; P=0.03).

Conclusion: Upregulation of miR-483-3p under high glucose conditions and in T2D may jeopardize a fast and efficient re-endothelialization response after acute vascular injury. This effect could result from two different mechanisms. While overexpression of miR-483 within endothelial cells can directly induce endothelial cell apoptosis, enhanced miR-483 levels in paracrinally active “accessory” cells, such as EOC, may affect their secretory support for the re-establishment of a homogenous endothelial layer. The repression of miR-483-3p levels in patients with T2D might therefore partially rescue the capacity of endothelial cells to mount a fast and efficient response to vascular injury.

CARDOVASCULAR IMPACT OF SOLUBLE EPOXIDE HYDROLASE INHIBITION IN A MURINE MODEL OF TYPE 2 DIABETES

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Inhibiting epoxyeicosatrienoic acid (EET) degradation by soluble epoxide hydrolase (sEH) improves glucose homeostasis in animal models of insulin resistance and type 2 diabetes. However, the impact of such strategy on diabetic cardiovascular complications remains to be clarified.

FVB mice subjected to a control chow diet (10% fat) or an high-fat diet (HFD, 60% fat) for 16 weeks. After 8 weeks, HFD mice received for the remaining 8 weeks the sEH inhibitor t-AUCB (10 mg/L in drinking water), the sulfonylurea glibenclamide (80 mg/L) or were not treated. Vascular reactivity was assessed by myography at the level of septal coronary arteries and cardiac geometry and function was evaluated by echocardiography and invasive hemodynamics.

Compared with control mice, HFD mice gained more weight and developed type 2 diabetes, as shown by their increase in fasting glycemia and insulinemia. Glibenclamide and t-AUCB similarly prevented the increase in fasting glycemia in HFD mice without affecting insulinemia. Compared with control mice, the coronary endothelium-dependent relaxations to acetylcholine were markedly reduced in non-treated HFD mice. The NO-synthase inhibitor L-NA decreased these relaxations in both groups but this decrease was lesser in non-treated HFD mice compared with control mice. Moreover, the addition of MSPPOH further decreased these relaxations in control mice but not in non-treated HFD mice. Finally, the relaxations to the NO donor sodium nitroprusside and to the openers of calcium-activated potassium channels, the cellular target of EETs mediating their hyperpolarizing effect, NS309 and NS1619 were decreased in non-treated HFD mice. None of these responses were improved by glibenclamide, while t-AUCB increased the relaxations to acetylcholine and slightly increased the inhibitory effect of L-NA on these relaxations compared with non-treated HFD mice, together with the improvement in the relaxations to sodium nitroprusside. In addition, t-AUCB fully restored the sensitivity to MSPPOH, without affecting the relaxations to NS309 and NS1619. Finally, t-AUCB decreased cardiac inflammation, fibrosis, and hypertrophy as well as improved diastolic dysfunction, demonstrated by the increased E/A ratio and decreased slope of the end-diastolic pressure-volume relation.

These results demonstrate that, independently from its glucose-lowering effect, the inhibition of sEH improves vascular dysfunction by restoring NO and EET pathway and prevents cardiac dysfunction in type 2 diabetes. This combined beneficial impact on glucose homeostasis and target organ damage may be useful to improve cardiovascular prognosis in diabetic patients.
1 Diabetes  p. 32–35
1/1 Ramesh Chennupati, Maastricht NL
1/2 Irina C. Chis, M.D., Cluj-Napoca RO
1/3 Cuiqing Liu, Ph.D., Hangzhou CN
1/4–1/5 Francesco Paneni, M.D., Zurich CH and Rome IT
1/6–1/7 Elena Zinssius, Mainz DE

2 Natural Products  p. 36–38
2/1–2/2 Cyril Auger, Ph.D., Illkirch FR
2/3 Yu Cai, Hong Kong CN
2/4 Rachel W.S. Li, Hong Kong CN
2/5 Heikki Vapaatalo, M.D., Ph.D., Helsinki FI

3 Pathology and Pharmacology  p. 37–51
3/1–3/2 Alexander Akhmedov, Ph.D., Zurich CH
3/3–3/6 Alexander E. Berezin, Ph.D., Zaporozhye UA
3/7–3/8 Julie Boisramé-Helms, M.D., Strasbourg FR
3/9 Daniel Gaul, M.Sc., Zurich CH
3/10 Daihiko Hakuno, M.D., Tokorozawa JP
3/11 Susanne Karbach, M.D., Mainz DE
3/12 Roland Klingenberg, M.D., Zurich CH
3/13 Chang Liu, M.D., Fribourg CH
3/14 Michael Mader, Mainz DE
3/15 Melroy X. Miranda, M.D., Zurich CH
3/16 Francesco Paneni, M.D., Zurich CH
3/17 Martin F. Reiner, M.D., Zurich CH
3/18 Remo D. Spescha, M.Sc., Zurich CH
3/19 Sebastian Steven, M.D., Mainz DE
3/20 Florence Toti, Ph.D., Illkirch FR
3/21 Yu Wang, Ph.D., Hong Kong CN
3/22 Zongsong Wu, Fribourg CH
3/23 Yuyan Xiong, Fribourg CH
3/24 Yi Yu, Fribourg CH

4 Vascular Responsiveness  p. 52–58
4/1–4/2 Jean-Luc Cracowski, Ph.D., Gernoble FR
4/3 Smita Dutta Roy, M.D., Gothenburg SE
4/4 Florence Gaillard-Bigot, M.Sc., Geroble FR
4/5 Rok Humar, M.D., Zurich CH
4/6 Tamas Kriska, Ph.D., Dallas USA
4/7 Susan Wai-Sum Leung, Ph.D., Hong Kong CN
4/8 Dharmani Devi Murugan, Ph.D., Kuala Lumpur MY
4/9 Miroslav D. Radenković, M.D., Belgrade RS
4/10 Holger Schneider, Ph.D., Munich DE
4/11 Nikolai D. Temnyalov, Ph.D., Varna BG
4/12 Yuming Wu, Ph.D., Shijiazhuang CN
ARGinine recycling is important to maintain vasomotor functions in diabetes

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Introduction: Arginine recycling by argininosuccinatesynthetase (ASS) influences the bio-availability of arginine. We assessed whether impaired arginine resynthesis reduces endothelium-dependent vasodilatation in normal and diabetic mice.

Methods: Endothelium-selective ASS-deficient mice (Assfl/fl/Tie2Cre-tg/-) were generated by crossing Assfl/fl mice with Tie2Cre mice. PCR and immunohistochemistry were performed to confirm the deletion of the enzymes. Mean arterial blood pressure (MAP) was recorded in conscious 34-week-old male mice using intra-arterial catheters. Vasomotor responses were studied in saphenous arteries of 12- and 34-week-old ASS-KO and floxed control animals (WT) by wire-myography. At the age of 10-weeks, mice were made diabetic by STZ (50 mg/kg, on 5 consecutive days) and vasomotor responses were then studied at 20 weeks of age.

Results: Basal MAP was similar in WT and ASS-KO mice. MAP was increased to the same extent in WT and ASS-KO by depletion of circulating arginine (200 U arginase1 intravenously) or inhibition of NOS activity (L-NAME, 10mg/kg). Optimal arterial diameter, contractile responses to phenylephrine, and relaxing responses to acetylcholine (ACh) and sodium nitroprusside did not differ between WT and ASS-KO. In diabetic ASS-KO, compared to diabetic WT mice, ACh-induced relaxation was significantly reduced in the absence and presence of indomethacin (INDO, 10 µM) and of both INDO and 40 mM K+. In the presence of both INDO and L-NAME (100 µM), relaxing responses to ACh and to Na-nitroprusside did not differ between the diabetic mouse strains.

Conclusions: Absence of endothelial arginine recycling from citrulline does not affect blood pressure and vasomotor responses in normal mice. Diabetes impairs vasodilatation mediated by endothelium-derived NO to a larger extent in ASS-KO than WT mice. This suggests that arginine recycling in the endothelium limits endothelial dysfunction in diabetes.

EFFECTS OF CHRONIC (TRAINING) EXERCISE ON OXIDATIVE STRESS STATUS IN AN ANIMAL MODEL OF TYPE 2 DIABETES MELLITUS

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Introduction: Increasing evidence in both experimental and clinical studies suggests that oxidative stress is involved in the pathogenesis and progression of diabetic tissue damage. This study investigated the effects of chronic exercise training associated with chronic oral administration of quercetin on blood glucose levels, vascular function and oxidative stress status in streptozotocin-induced diabetic Wistar rats.

Methods: Adult male Wistar rats were divided into seven groups: Group I: non-diabetic, sedentary control rats; Group II: non-diabetic, trained control rats; Group III: non-diabetic, trained control rats treated with quercetin; Group IV: diabetic, sedentary control rats; Group V: diabetic, trained control rats; Group VI: diabetic, sedentary rats treated with quercetin; Group VII: diabetic, trained rats treated with quercetin. Quercetin was administered via an intragastric tube (0.6 ml/rat), at a dose of 20 mg/kg body weight/day for 4 weeks after the induction of diabetes mellitus. Diabetes was induced by a single i.p. injection of streptozotocin (40 mg/kg body weight). Animals were sacrificed at the end of a 4-week swimming training program. The glycemic profile, oxidative status (lipid peroxidation and protein oxidation), antioxidant levels (catalase, SOD and glutathione peroxidase) and the serum NO (measured by ELISA) were evaluated.

Results: When compared to diabetic sedentary rats, the animals submitted to chronic exercise presented significantly lower glycaemic values, and significantly increased oxidative stress levels. The diabetic, trained rats treated with quercetin presented significantly lower glycaemic values accompanied by a remarkable reduction of oxidative markers as well as a decrease of NO degradation.

Conclusion: The results suggested that chronic exercise training associated with quercetin administration could lower blood glucose levels, restore vascular function and reduce oxidative stress in type 2 diabetic rats.
TARGETING OF RHO KINASE AMELIORATES IMPAIRMENT OF DIABETIC ENDOThelial FUNCTION IN INTRARENAL ARTERY

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Introduction: Endothelial dysfunction in kidney vasculature is the initial and key element for nephropathy in diabetes mellitus. Accumulating evidence suggests the protective role of Rho kinase inhibitors in endothelial dysfunction via modulating eNOS activity and NO production. However, the role of Rho kinase in diabetes-related endothelium dysfunction in kidney vasculature and the relevant mechanisms remain unknown. Here, we assessed whether pharmacological inhibition of Rho kinase attenuates endothelial dysfunction in intrarenal arteries using type 1 diabetic rats.

Method: Diabetes was induced by intraperitoneally injecting streptozotocin with male Sprague-Dawley rats. Three days after the verification of diabetes, diabetic rats were treated with fasudil (Rho kinase inhibitor, 10 mg/kg/day) for 4 weeks. Intrarenal arteries were isolated and suspended in myograph for the measurement of changes in isometric tension. Histology and mesangial expansion in the kidney were examined with Periodic Acid Schiff staining. Rho kinase protein levels and activity in renal cortex were assessed with western blot and immunohistochemistry respectively. Superoxide in the renal artery was measured with DHE staining and mRNA level of NADPH was determined with quantitative PCR.

Results: Fasudil, a Rho kinase inhibitor effectively decreased the phosphorylated level of MYPT1 without affecting the expression of ROCK I or ROCK II in the kidney. Fasudil treatment showed no improvement in diabetes-related abnormality in metabolic indices, but it significantly ameliorated endothelial dysfunction in intrarenal arteries, evidenced by both enhancement in acetylcholine-induced vasodilatation and inhibition in acetylcholine-induced vasoconstriction in presence of L-NAME. The treatment with fasudil also lessened the mesangial matrix expansion in the kidney cortex. Mechanistically, superoxide production in the intrarenal artery and NOX4 subunit of NADPH oxidase in the renal cortex that contribute to diabetic nephropathy were also prevented by the Rho kinase inhibitor.

Conclusion: In conclusion, the present results indicate that Rho kinase is involved in endothelial dysfunction in type 1 diabetes via enhancement of oxidative stress and provides new evidence for Rho kinase inhibitors as potential therapeutic agents for the treatment of diabetic nephropathy.

1/3

EPIGENETIC SIGNATURES INDUCED BY THE ACETYLTRANSFERASE GCN5 MEDIATE OXIDATIVE STRESS AND ENDOThelial DYSFUNCTION IN DIABETES

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Introduction: Epigenetic modifications are recently emerging as important modulators of gene expression. The mammalian acetyltransferase gene non-derepressible 5 (GCN5) modulates genes involved in growth and survival through acetylation of histones. Whether GCN5 participates to a cardiovascular disease phenotype remains to be determined. The present study investigates GCN5 role in diabetes-related vascular dysfunction.

Methods: Human aortic endothelial cells (HAECs) were exposed to normal (NG, 5mmol/L) or high glucose concentrations (HG, 25 mmol/L) in the presence or in the absence of GCN5 pharmacological inhibitor CPTH2 or siRNA-mediated knockdown. Superoxide anion (O2-·) was measured by ESR spectroscopy. A custom array including 20 oxidative genes affected by hyperglycemia was performed with or without GCN5 inhibition. In parallel, 30 patients with type 2 diabetes (T2DM) and 18 age-matched healthy controls were enrolled. All patients underwent flow-mediated vasodilation (FMD) of the brachial artery to assess endothelial function. Urinary levels of 8-isoprostaglandinF2α (8-isoPGF2α) were measured as a marker of oxidative stress. Gene expression of GCN5, manganese superoxide dismutase (MnSOD), Nox2 and p66Shc was assessed from peripheral blood monocytes. Data are presented as fold change (FC) or percentage of control.

Results: HG significantly increased GCN5 expression in HAECs (155±20% vs. NG, p<0.05). MnSOD expression was blunted by HG (FC vs. NG: -3.4, p<0.05) while NADPH subunit Nox2 as well as the mitochondrial adaptor p66Shc were significantly upregulated (FC vs. NG: 9.7 and 2.5, respectively, p<0.05). Interestingly, GCN5 inhibition or siRNA prevented hyperglycemia-induced deregulation of oxidative genes and O2-· increase, suggesting its pivotal role in modulating endothelial oxidative stress response in this setting. GCN5 expression was also increased in PBMC of T2DM patients (455±155% vs controls p=0.05) and significantly correlated with MnSOD (r=-0.29, p>0.05), Nox2 (r=0.32, p<0.05), and p66Shc (r=0.49, p<0.05). Consistently, GCN5 upregulation paralleled endothelial dysfunction (r=-0.33, p<0.05) and oxidative stress (r=0.46, p<0.05), as assessed by FMD and 8-isoPGF2α, respectively.

Conclusions: GCN5 mediates hyperglycemia-induced oxidative stress via epigenetic regulation of oxidant and ROS scavenger enzymes. The relevance of these findings is supported by the observation that the acetyltransferase is overexpressed in human diabetes and correlates with oxidative stress and vascular dysfunction. Our findings provides a novel therapeutic opportunity for the prevention of diabetes-related vascular oxidative stress.
TARGETING PROLYL-ISOMERASE-1 SUPPRESSES MITOCHONDRIAL OXIDATIVE STRESS AND VASCULAR INFLAMMATION: ALTERATIONS IN PATIENTS WITH TYPE 2 DIABETES

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Introduction: Prolyl-isomerase-1 (Pin1) regulates function of protein substrates through isomerization of peptide bonds that link phosphoserine or phosphothreonine to proline. Pin1 triggers reactive oxygen species (ROS) production and inflammation in human cancer. Whether Pin1 is involved in cardiovascular disease remains largely unknown. This study investigates the role of Pin1 in diabetes-related vascular dysfunction.

Methods: Human aortic endothelial cells (HAECs) were exposed to normal (NG, 5 mmol/L) or high glucose concentrations (HG, 25 mmol/L) in the presence or in the absence of Pin1 pharmacological inhibitor Juglone or siRNA-mediated knockdown. Diabetes was induced in C57/B6 mice (aged 4-6 months) by streptozocin and animals treated i.v with Pin1 siRNA or Juglone i.p for 30 days. Endothelial function was assessed by dose-response curve with acetylcoline. Protein expression was assessed by immunoblotting. Mitochondrial ROS were measured by ESR spectroscopy. Immunoprecipitation was performed to show the interaction of Pin1 with phosphorylated p66Shc and NFkB subunit p65. Interestingly, Juglone or Pin1siRNA prevented p66 Shc-induced ROS production and suppressed upregulation of adhesion molecules VCAM-1, ICAM-1 and MCP-1.

Results: Pin1 expression markedly increased in HAECs exposed to HG (289±22% vs. NG, p<0.01) and aortas of diabetic mice (216±32 vs. controls, p<0.05). Immunoprecipitation showed that Pin1 recognizes phosphoserine motifs of the pro-oxidant mitochondrial adaptor p66Shc as well as NFkB subunit p65. Interestingly, Juglone or Pin1siRNA prevented p66Shc-induced ROS production and suppressed upregulation of adhesion molecules VCAM-1, ICAM-1 and MCP-1 via inhibition of p65 nuclear translocation. In vivo knockdown of Pin1 or Juglone in diabetic mice protected against hyperglycemia-induced endothelial dysfunction, ROS production and vascular inflammation. Of note, Pin1 mRNA was significantly upregulated in PBM of T2DM patients as compared with healthy controls (370±97 vs. 25±28, p<0.01) and correlated with glycated haemoglobin (r=0.44, p<0.05), flow-mediated vasodilation (FMD) (r=-0.36, p<0.01), urinary 8-isoPGF2α (r=0.39, p<0.05), VCAM-1 (r=0.56, p<0.05) and ICAM-1 (r=0.53, p<0.05).

Conclusions: This study shows for the first time that Pin1 may critically participate to oxidative stress burden, endothelial dysfunction and vascular inflammation in patients with type 2 diabetes.

EFFECTS OF TELMISARTAN OR AMLODIPINE MONOTHERAPY VERSUS TELMISARTAN/AMLODIPINE COMBINATION THERAPY ON VASCULAR DYSFUNCTION AND OXIDATIVE STRESS IN DIABETIC RATS

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Introduction: Our previous studies identified potent antioxidant effects and improvement of vascular function by telmisartan therapy in experimental diabetes and nitrate tolerance. The present study compared the beneficial effects of single telmisartan or amlodipine versus telmisartan/amlodipine combination therapy (T+A) in streptozotocin (STZ)-induced type 1 diabetic rats.

Methods: Male Wistar rats were injected once with STZ (60 mg/kg, i.v.) and one week later the drugs (telmisartan, amlodipine or T+A) were administered orally by special diet (2.5-5 mg/kg/d) for another 7 weeks.

Results: We only observed a marginal beneficial on-top effect of T+A therapy over the single drug regimen that was most evident in the improvement of endothelial function (acetylcholine response) and less pronounced in the reduction of whole blood, vascular and cardiac oxidative stress (blood leukocyte oxidative burst, aortic dihydroethidine and 3-nitrotyrosine staining as well as cardiac NADPH oxidase activity and uncoupling of endothelial nitric oxide synthase) in diabetic rats. These effects on oxidative stress parameters were mimicked by the expression pattern of NADPH oxidase and nitric oxide synthase isoforms. In addition, development of mild hypotension in the T+A treated rats was observed.

Conclusions: Reasons for this moderate synergistic effect of T+A therapy may be related to the potent beneficial effects of the telmisartan alone and the fact that amlodipine and telmisartan share similar pathways to improve endothelial function. Moreover, hypotension in the T+A treated rats could partially antagonize the beneficial additive effects by counter-regulatory mechanisms (e.g. activation of the renin-angiotensin-aldosterone-system [RAAS]).
EFFECTS OF EMPAGLIFLOZIN ON OXIDATIVE STRESS AND ENDOTHELIAL DYSFUNCTION IN STREPTOZOTOZIN-INDUCED TYPE I DIABETIC RAT

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Introduction: In diabetes, cardiovascular complications are associated with endothelial dysfunction and oxidative stress. Empagliflozin (Empa), as a selective sodium glucose cotransporter 2 inhibitor (SGLT2) in clinical development, offers a promising novel approach for the treatment of type 2 diabetes by enhancing urinary glucose excretion. The aim of the present study was to test whether treatment with Empa could improve endothelial dysfunction in type 1 diabetic rats via reduction of glucotoxicity and associated oxidative stress.

Methods: Type I diabetes in Wistar rats was induced by an intravenous injection of streptozotocin (60 mg/kg). One week after injection Empa was administered via drinking water for 7 weeks.

Results: Treatment with Empa (10 and 30 mg/kg/d), showed reduction of blood glucose and a normalization of endothelial dysfunction (aortic rings) in diabetic rats and a reduced oxidative stress in aortic vessels (dihydroethidine staining), in blood (phorbol ester/zymosan A-stimulated chemiluminescence). Additionally, the higher NADPH-oxidase activity in heart tissue of diabetic animals was normalized by SGLT2i therapy.

Conclusion: In this study we could demonstrate that Empa improves hyperglycemia and prevents the development of endothelial dysfunction and oxidative stress in type 1 diabetic rats. Future studies will investigate the underlying mechanisms of these antioxidant and anti-inflammatory effects with special emphasis on the activity of NADPH oxidase and the prevention of uncoupling of the nitric oxide synthase, which contributes to cardiovascular complications.
THE NO-MEDIATED RELAXATION INDUCED BY THE HIGHLY PURIFIED EPA:DHA 6:1 PRODUCT INVOLVES A COPPER-DEPENDENT REDOX-SENSITIVE ACTIVATION OF THE PI3-KINASE/AKT PATHWAY LEADING TO eNOS ACTIVATION

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Introduction: Omega-3 fatty acid products containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to reduce the risk of cardiovascular disease, in part, by stimulating the endothelial formation of nitric oxide (NO), a potent vasoprotective factor. The aim of the present study was to determine whether the EPA:DHA ratio and purity affect the ability to cause endothelial-dependent relaxations in arterial rings, and to characterize the mechanism leading to endothelial NO synthase (eNOS) activation.

Methods: The reactivity of porcine coronary artery rings was assessed in organ chambers, the expression and phosphorylation levels of proteins in cultured porcine coronary artery endothelial cells by Western blot analysis, and vascular formation of reactive oxygen species (ROS) in coronary artery sections using dihydroethidium and confocal microscopy.

Results: EPA:DHA 6:1 caused significantly greater endothelium-dependent relaxations in porcine coronary artery rings than EPA:DHA 1:1, EPA and DHA alone, and EPA:DHA 6:1 with a reduced EPA + DHA amount. Relaxations to EPA:DHA 6:1 were slightly but significantly reduced by an eNOS inhibitor, not affected by inhibition of endothelial-dependent hyperpolarization and abolished by both treatments. Relaxations to EPA:DHA 6:1 were insensitive to cyclooxygenase inhibition, and reduced by membrane permeant chelators of ROS (MnTMPyP and PEG-catalase), inhibitors of either Src kinase or PI3-kinase, and copper chelating agents. Moreover, the relaxations were significantly reduced by inhibitor of either p38 MAPK, JNK, and MEK, but not by an inhibitor of AMPK. In cultured endothelial cells, EPA:DHA 6:1 induced phosphorylation of activating sites of Src (Tyr418), Akt (Ser473) and eNOS (Ser 1177); these effects were inhibited by MnTMPyP and PEG-catalase. In coronary artery sections, EPA:DHA 6:1 induced the formation of ROS in the endothelium but not the vascular smooth muscle, and this effect was inhibited by MnTMPyP, PEG-catalase, and intracellular copper chelating agents.

Conclusion: Omega-3 fatty acids cause endothelium-dependent NO-mediated relaxations in coronary artery rings, which are dependent on the EPA and DHA ratio and purity. Moreover, the NO-mediated relaxation involves an intracellular copper-dependent event triggering the redox-sensitive activation of JNK/p38MAPK/MEK and the PI3-kinase/Akt pathway leading to the subsequent activation of eNOS by phosphorylation at Ser 1177.

ORAL INTAKE OF BLACKCURRANT JUICE PREVENTS ENDOTHELIAL DYSFUNCTION IN THE MESENTERIC ARTERY OF CIRRHOTIC RATS WITH PORTAL HYPERTENSION

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Introduction: Chronic liver diseases with portal hypertension (PH) are characterized by a progressive vasodilatation associated with endothelial dysfunction, which is especially observed in the splanchnic and pulmonary beds. The latter is referred to as the hepatopulmonary syndrome (HPS).

Methods: Male Wistar rats (8 rats per group) received either control drinking water or a polyphenol-rich Blackcurrant solution (60 mg total phenols/kg) for 7 weeks. After 3 weeks, the rats underwent surgery with either the ligation and resection of the common bile duct (CBDL rats) or sham surgery (sham rats), and, then, they were followed for 4 weeks. Reactivity of mesenteric artery rings was assessed in organ chambers, the expression level of proteins in the mesenteric artery and/or aorta by immunofluorescence, the vascular formation of reactive oxygen species (ROS) using dihydroethidium, and plasma levels of pro-inflammatory cytokines including TNF-α, IL-1β, MCP-1 and IL-6 by flow cytometry using a commercial kit.

Results: Both the NO- and the EDH-mediated relaxations to aceetylcholine were significantly reduced in CBDL rats compared to sham rats, whereas relaxations to sodium nitroprusside (an exogenous donor of NO) and levromakalim (an ATP-sensitive K+ channel opener) were similar. The endothelial dysfunction was associated with a reduced vascular expression of CX37 and SKCa and an increased expression of eNOS. In aortic sections, an increased expression of NADPH oxidase subunits (p22phox, p47phox) and vascular formation of ROS and peroxynitrite were observed in the CBDL group. The PRBJ treatment improved blunted EDH-mediated relaxation, but not the NO-mediated relaxation induced in CBDL rats, and this effect was associated with an improved vascular expression of CX37 and SKCa, and eNOS. The PRBJ treatment also reduced vascular oxidative stress in the aorta. Moreover, the CBDL-induced increased plasma levels of pro-inflammatory cytokines were improved.

Conclusion: Altogether, these findings indicate that CBDL induced an endothelial dysfunction affecting both NO and EDH components, and that this effect is associated with increased vascular oxidative stress and inflammation. Chronic ingestion of PRBJ improved the CBDL-induced blunted EDH-mediated relaxation, most likely by normalizing the vascular oxidative stress and the inflammatory response.
MODULATION OF SIRT1 ACTIVITY IN ENDOTHELIAL CELLS AFFECTS THE THERAPEUTIC EFFECTS OF RESVERATROL IN APOLIPROTEIN E DEFICIENT MICE

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Introduction: Sirtuin-1 (SIRT1) possesses anti-metabolic and vascular aging properties, and elicits protective functions against atherosclerosis and metabolic abnormalities. Resveratrol, one of the activator of SIRT1, performs therapeutic effects on cardiovascular and metabolic homeostasis through SIRT1-dependent and SIRT1-independent pathway. The present study aimed to investigate the therapeutic effects of resveratrol in hypercholesterolemic apolipoprotein E-deficient (Apoe-/-) mice with different endothelial SIRT1 expression and activity levels.

Method: ApoE-/- mice with endothelial-selective overexpression of wild type human SIRT1 (hSIRT1) (WIS ApoE-/-) or its dominant negative mutant hSIRT1 (H363Y) (HIS ApoE-/-) under high fat high cholesterol diet were administered with resveratrol for eight weeks. Serum and tissues were collected for lipid profiling and histological analysis. SIRT1 activity was evaluated by western blot analysis and in-vitro deacetylation assay.

Results: Resveratrol significantly improved serum and liver lipid profiles and attenuated atherosclerotic lesions in both ApoE-/- and HIS ApoE-/- mice. However, it adversely affected serum and liver lipid profiles in WIS ApoE-/- mice. Resveratrol did not significantly affect SIRT1 activity in liver tissues of all mice groups.

Conclusion: The protected effects on cardiovascular and metabolic homeostasis of resveratrol in ApoE-/- mice were modulated by SIRT1 activity in endothelial cells.

VASODILATORY EFFECT OF NARIRUTIN IN RAT MESENTERIC ARTERIES AND AORTAE

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Introduction: Narirutin, a kind of flavanones, is abundant in citrus fruits. It has been shown to possess anti-inflammatory and anti-oxidative effects. Its structural similarities, naringin and naringenin, were found to be vasoprotective. However, the vasoprotective effect of narirutin has not yet been reported. This study aimed to investigate the vasodilatory effect of narirutin.

Method: Male Sprague-Dawley (SD) rats (8-10 weeks old), spontaneously hypertensive rats (SHR) (36-40 weeks old) and the normotensive controls (Wistar-Kyoto rats; WKY) were used in this study. The mesenteric arteries and aortae were isolated for the measurement of isometric tension in relaxation and contraction studies, respectively.

Results: The results showed that narirutin caused a concentration-dependent dilation of mesenteric arteries in SD rats. The vasodilation was stronger when the endothelium was present and this effect was partially blocked by L-NAME (a nitric oxide synthase inhibitor), ODQ (a soluble guanylyl cyclase inhibitor) or 4-aminopyridine (a voltage-activated K+ channel blocker). Besides, the impaired acetylcholine (Ach) induced-dilation of mesenteric arteries in SHR was restored when the arteries were pre-incubated with narirutin (10 µM and 30 µM) for 30 minutes. This effect was not observed in the mesenteric arteries of WKY. Furthermore, the Ach-induced endothelium-dependent contraction was studied in the aorta of SHR in the presence of L-NAME. The Ach-induced vasoconstriction in SHR was decreased and even abolished when the aortae were pre-incubated with 10 µM and 30µM of narirutin, respectively.

Conclusion: This study demonstrated that the direct vasodilation induced by narirutin was partially endothelium-dependent, which may involve the participation of nitric oxide and voltage-activated K+ channels. Besides, narirutin was able to restore the impaired endothelium-dependent vasodilation and augmented endothelium-dependent vasoconstriction in hypertensive rat model.
PEPTIDES, BERRIES AND VASCULAR FUNCTION

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Introduction: Functional foods containing small bioactive peptides and berries rich in polyphenols are suggested for prevention of cardiovascular problems such as slightly or moderately increased blood pressure and development of vascular stiffness. Tripeptides Isoleucine-Proline-Proline (IPP) Valine-Proline-Proline (VPP) show angiotensin converting enzyme (ACE-1) inhibiting properties while polyphenols act mainly as antioxidants reducing reactive oxygen species and minimal endothelial inflammation seen in hypertension. The two prolines in the tripeptide structure can rotate, and the peptide can exist in different cis-trans-configuration and thus fit with different affinity to the active site of the enzymes concerned.

Methods: Spontaneously hypertensive male rats (SHR) were used to test the antihypertensive properties (tail-cuff method) of the peptide products and berry juices in prolonged treatments. Mesenteric artery and aortic rings from these animals were used to investigate changes in vascular function in vitro, or the peptide effect was tested by dosing the compound directly into the incubation of the vascular rings. The AutoDock 4.2. docking software was used to predict the suitable peptide bond configurations on two enzymes ACE-1 and cyclooxygenase-2 (COX-2) in silico.

Results: Milk based products containing the two tripeptides both prevented the development of hypertension and lowered already developed hypertension in SHR in a 6-8 week treatment. Endothelium dependent vascular relaxation was improved by the peptide treatment. Incubation of the mesenteric artery rings in the presence of IPP preserved endothelial function. Bradykinin-induced endothelium dependent vascular relaxation was age-related and IPP potentiated this action via Ang(1-7)-Mas-receptor axis. Inhibition of bradykinin receptors (B1 and B2) or ACE-1 inhibition did not modify the relaxation suggesting other mechanisms than the classic ones. Of the berries, tested lingonberry juice proved to be the most potent in prevention of hypertension of SHR or preserving endothelial function in long term feeding depending of the dosage. These effects were related to reduction of ACE-1, COX-2, monocyte chemotactic protein (MCP-1) and p-selectin expressions. Modelling of peptide structure showed that cis-cis configuration is the most favourable structure fitting ACE-1 while in COX-2 modelling no such preference was found.

Conclusions: Prolonged feeding of spontaneously hypertensive rats with milk based products containing bioactive tripeptides or lingonberry juice alone showed an antihypertensive effect. The treatments preserved endothelial function independently of blood pressure changes. Beneficial vascular effects of the peptides in vitro may be mediated via bradykinin -(Ang1-7) -Mas-receptor axis. The proline-proline configuration is an interesting option for further studies.
ENDOTHELIAL OVEREXPRESSION OF LOX-1 INCREASES STROKE SIZE IN VIVO
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Background- Stroke is one of the most common causes of death and long term disability worldwide primarily affecting the elderly population. Lectin-like oxidized LDL receptor 1 (LOX-1) is the receptor for oxidized LDL identified in endothelial cells. Binding of OxLDL to LOX-1 induces several cellular events in endothelial cells, such as activation of transcription factor NF-kB, upregulation of MCP-1, and reduction in intracellular NO. Accumulating evidence suggests that LOX-1 is involved in endothelial dysfunction, inflammation, atherogenesis, myocardial infarction, and intimal thickening after balloon catheter injury. Interestingly, a recent study demonstrated that acetylsalicylic acid (aspirin), which could prevent ischemic stroke, inhibited OxLDL-mediated LOX-1 expression in human coronary endothelial cells. The expression of LOX-1 was increased at a transient ischemic core site in the rat middle cerebral artery occlusion model. These data suggest that LOX-1 expression induces atherosclerosis in the brain and is the precipitating cause of ischemic stroke. Therefore, the goal of the present study was to investigate the role of endothelial LOX-1 in stroke using experimental mouse model.

Methods and Results- 12-week-old male LOX-1TG generated recently in our group and wild-type (WT) mice were applied for a transient middle cerebral artery occlusion (MCAO) model to induce ischemia/reperfusion (I/R) brain injury. LOX-1TG mice developed 24h post-MCAO significantly larger infarcts in the brain compared to WT (81.51±8.84 vs. 46.41±10.13, n=7, p < 0.05) as assessed morphologically using Triphenyltetrazolium chloride (TTC) staining. Moreover, LOX-1TG showed higher neurological deficit in RotaRod (35.57±8.92 vs. 66.14±10.63, n=7, p < 0.05) and Bederson tests (2.22±0.14 vs. 1.25±0.30, n=7 vs. 12, p < 0.05) – two experimental physiological tests for neurological function.

Conclusions- Thus, our data suggest that LOX-1 plays a critical role in the ischemic stroke when expressed at unphysiological levels. Such LOX-1-associated phenotype could be due to the endothelial dysfunction. Therefore, LOX-1 may represent a novel therapeutic target in the early phase of myocardial infarction.

GENETIC DELETION OF p66SHC ADAPTOR PROTEIN LEADS TO INCREASED MYOCARDIAL INFARCTION BY INHIBITING RISK AND SAFE PROSURVIVAL PATHWAYS
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Background- Formation of reactive oxygen species (ROS) contributes to many pathophysiological processes. Although ROS production is also involved in some physiological processes, the imbalance between their generation and removal, i.e. oxidative stress, plays a major role in particular in myocardial injury caused by ischemia-reperfusion (I/R). The mammalian Shc locus encodes three Shc isoforms: p46Shc, p52Shc and p66Shc. The p66Shc is not involved in mitogenic signals as p46Shc/p52Shc, but it functions as a critical mediator of intracellular oxidative signal transduction. Various studies relate p66Shc to cardiovascular disease; however, few data are available on the role of p66Shc in myocardial I/R.

Methods and Results- 8-12-week-old male p66Shc deficient (p66Shc−/−) mice and corresponding C57Bl/6 wild-type (WT) control mice were subjected in vivo to different durations of ischemia (30, 45 and 60 min) followed by 24h of reperfusion. Infarct size was assessed morphologically and by MRI. After 30 min of ischemia, p66Shc−/− mice developed markedly larger infarcts as compared to WT (infarct size [I]/area at risk [AAR]: 20.46±5.02 % vs. 7.72±1.31%, n=12-14, p < 0.05). This effect was confirmed by in vivo silencing of p66Shc prior to I/R. Both genetic deletion and silencing of p66Shc displayed an increased post-ischemic levels of serum cardiac troponin I (cTnI). However, the observed effect on infarct size was limited to 30 min of ischemia since by increasing ischemia duration to either 45 or 60 min infarct size did no longer differ between p66Shc−/− and WT mice. Moreover, differently from WT, infarct size in p66Shc−/− was not significantly larger with increasing duration of ischemia (from 30 to 60 min). On the molecular level the observed effect was linked to the inhibition of activation by phosphorylation of protein kinase Akt and transcription factor STAT3 – two key members of prosurvival pathways RISK and SAFE, respectively.

Conclusions- Our data suggest that genetic deletion of p66Shc leads to an increased sensitivity to myocardial infarction with larger infarcts with shorter, but not prolonged ischemia, and that RISK and SAFE prosurvival pathways are involved. Therefore, activation of p66Shc may provide resistance to ischemia and represent a novel therapeutic target in the early phase of myocardial infarction.
CIRCULATING ENDOTHELIA PROGENITOR CELLS IN CHRONIC HEART FAILURE PATIENTS WITH CORONARY ARTERY DISEASE

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Objective: of this study was to evaluate an association between severity of left ventricular dysfunction and circulating endothelial progenitor cells, proangiogenic monocytes CD45−CD34+CD14+CD309+ and CD14+CD309+ Tie2+ in chronic heart failure patients (CHF) with stable coronary artery disease (CAD).

Methods: 126 subjects (54 male) aged 48-62 years with mild-to-moderate CHF (median of left ventricular ejection fraction [LVEF] was 47.7%; 95% confidence interval was 41.8 to 54.3%) due to CAD and 25 healthy volunteers were enrolled in the study. Vessel-wall and plaque geometrical and compositional parameters were measured on contrast-enhanced computer tomography angiography. Coronary calcification was graded by calculating the Agatston score index. Immunostaining and flow cytometric technique (FCT) was used for predictable distinguish cell subsets depended on the expression of CD14, CD34, Tie-2, CD45, and CD309 (VEGFR2). Circulating EPCs are defined as CD34/CD309 positive cells in lack CD45 expression. 500,000 events were analyzed from each tube. Standardized cell counts were presented as a percentage of total leukocytes, which were identified as the total number of all CD45+ cells.

Results: Analysis of obtaining outcomes has been shown a trend to decreasing of circulating CD45−CD34+ EPCs and a significantly reduction of EPCs population determined as CD14+CD309+ and CD14+CD309+ Tie2+ in CHF patients when compared with healthy volunteers. No significant associations between CD45−CD34+ and conventional cardiovascular risk factors in patient cohorts were found. Concentrations of CD14+CD309+ and CD14+CD309+ Tie2+ EPCs were contributed thereby such factors as LVEF (RR=2.86; 95% CI=1.90-6.20; P=0.002), high sensitive-C-reactive protein >10.12 mg/L (RR=2.14; 95% CI=1.90-3.70; P=0.009), functional class of CHF (RR=1.80; 95% CI=1.32-3.11; P=0.006), type 2 diabetes mellitus (RR=1.21; 95% CI=1.10-2.40; P=0.008).

Conclusion: The reduction of circulating proangiogenic monocytes determined as CD14+CD309+ and CD14+CD309+ Tie2+ is more significantly marker of CHF severity than EPCs in CHF patients with known stable CAD.

SERUM URIC ACID AS A MARKER OF CORONARY ATHEROSCLEROSIS IN ASYMPTOMATIC PATIENTS WITH KNOWN CORONARY ARTERY DISEASE

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Objective: to evaluate the interrelation between serum uric acid and coronary artery calcification in asymptomatic CAD subjects with preserved left ventricular systolic function.

Design and Methods: 126 subjects with previously documented asymptomatic coronary artery disease (CAD) were enrolled to the study. CAD was determined with contrast multispiral CT-angiography. SUA were measured enzymatic methods.

Results: Analysis of the results showed that mean value of SUA level was 23.84 mmol / L (95% CI = 15.75 – 31.25 mmol / L). In multivariate Cox regression analysis, the results showed that SUA levels (odds ratio [OR] = 1.42; 95% CI = 1.20 – 1.82; P <0.001), OPN (OR = 1.14; 95% CI = 1.12–1.25; P <0.001), OPG (OR = 1.45; 95% CI = 1.20–1.89; P <0.001), T2DM (OR = 1.41; 95% CI = 1.20–1.72; P <0.001), and TC (OR = 1.13; 95% CI = 1.10–1.22; P <0.001) were factors that independently associated with coronary artery calcification. After adjusting SUA level for OPN, OPG, T2DM, TC, demographic variables, it became to predict Agatston’ score index value (HR = 1.12; 95% CI = 1.01–1.52; P <0.001). The Cox models suggested that high quartile SUA level was very significant predictors of Agatston’ score index (Wald χ²= 147.83; P <0.001). The cutoff point of SUA for this model was 459 mmol / L. The sensitivity and specificity of the models were 80.0% and 59.2% respectively for predicting coronary artery calcification; AUC (Aria under curve) was 0.672±0.74. When SUA was added to these models as first, second, and third quartiles, it failed to predicting for Agatston’ score index (Wald χ² 14.28; P =0.661; Wald χ² 16.50; P =0.680; and Wald χ² 21.20; P =0.116 respectively).

Conclusions: We suggested that high quartile SUA level (cutoff point equaled 35.9 mmol / L) was very significant predictors of coronary calcification examined by Agatston’ score index in asymptomatic CAD subjects with preserved left ventricular systolic function.
PREDICT VALUE OF HIGH-SENSITIVE C-REACTIVE PROTEIN IN ARTERIAL HYPERTENSION PATIENTS AFTER ISCHEMIC STROKE

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Objective: To evaluate the relationship between high sensitive C-reactive protein (hs-CRP) plasma level and risk of recurrent coronary and cerebral ischemic events in arterial hypertension patients after ischemic stroke.

Method: 102 mild-to-moderate arterial hypertension patients (67 male, 56-68 aged) were enrolled to the study. Serum high-sensitivity CRP level was determined study entry only. Clinical interviews were performed every 3 months during 1 year after blood sampling. Clinical events included the following: certainly diagnosed ischemic stroke or TIA; coronary ischemic events, sudden death, diabetes mellitus, and all cardiovascular events including chronic heart failure and hospitalization.

Results: Patients in the highest quartile of hs-CRP level had a significantly higher adjusted odds ratio for clinical events when compared with those in the first quartile (odds ratio = 7.46; 95% CI = 1.55 - 19.6; P=0.001). A ROC curve detected a cutoff point of hs-CRP level of 5.58 mg/L (76.7% sensitivity, 80.3% specificity). Cox regression model identified elevated hs-CRP level that is exaggerated 5.58 mg/L as an independent predictor of further cardiovascular events (HR=7.14; 95% CI=1.15-12.6; P=0.009). Kaplan-Meier curves in Figure show that there was a significantly lower proportion of patients with hs-CRP <5.57 mg/L (Q1) with new clinical end point events (P=0.012) when compared with other quartiles of circulating hs-CRP levels. Figure. Kaplan-Meier estimates of the proportion of patients with cumulative clinical events depends on hs-CRP concentration by quartiles (Q).

In conclusion, we suggested that increasing of hs-CRP levels more than 5.58 mg/L strongly predicts the risk for cumulative clinical cardiovascular events in hypertensive patients after ischemic stroke.

CIRCULATING ENDOTHELIAL PROGENITOR CELLS IN PATIENTS WITH ISCHEMIC CHRONIC HEART FAILURE

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Introduction: The aim of this study was to evaluate circulating endothelial progenitor cells (EPC) level phenotyped as CD45-CD34+, CD14+CD309+ and CD14+CD309+Tie2+ in patients with ischemic chronic heart failure with preserved left ventricular ejection fraction.

Methods: 153 patients (86 men) aged 48-62 years with angiographically proven coronary artery disease in the presence of stenotic lesions of at least one coronary artery> 50%, and 25 healthy volunteers were included in the study. Ischemic chronic heart failure (CHF) was determined in 109 patients (65.0%) using traditional criteria in accordance with current clinical guideline. Phenotyping of mononuclear cells was performed by flow cytometers using monoclonal antibodies labeled with fluorochromes. Circulating EPC were defined as CD45CD34+. In order to identify subpopulations of EPA co-expressing CD14 antigen is further defined antigens both CD309 (VEGFR2) and Tie-2 antigens.

Results: It has found a negative impact of traditional cardiovascular risk factors such as type 2 diabetes, hyperlipidemia, hypertension, adherence to smoking toward to reducing of the circulating endothelial progenitor cells as hematopoietic as well as non-hematopoietic origin in patients with documented coronary artery disease irrespective of the chronic heart failure presentation. The decrease in the concentration of circulating endothelial progenitor cells with the phenotype CD14+CD309+ and CD14+CD309+Tie2- is associated with the severity of contractile and relaxation of myocardial dysfunction of the left ventricle, whereas the level of mononuclear cells with the phenotype CD45+CD34+ and CD45+CD34+Tie2+ to a greater extent reflects the prevalence and severity of atherosclerotic lesions of the coronary arteries.

Conclusion: There is predicted value of reduced level of circulating endothelial progenitor cells for increased chronic heart failure functional class, left ventricular ejection fraction < 42%, concentration of NT-pro-BNP > 554 pg/ml, E/Em ratio > than 15 units in ischemic chronic heart failure patients.
ENDOTHELIAL-DERIVED MICROPARTICLES CONTRIBUTE TO DISSEMINATED INTRAVASCULAR COAGULOPATHY DURING SEPTIC SHOCK

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Introduction: Septic shock-induced disseminated intravascular coagulopathy (DIC) contributes to endothelial dysfunction, multiple organ failure and high mortality rate. The objective of this study was to assess the potential role of procoagulant microparticles (MPs) as time course strong biomarkers of vascular cells contribution to DIC.

Patients and Methods: Prospective multicenter study on one hundred consecutive adult patients with septic shock enrolled at admission in medical intensive care units. Hemostasis parameters were analyzed and microparticles measured by prothrombinase assay after capture onto insolubilized annexin-5 or biotynilated antibodies to determine their cell origin.

Results: 92 patients were analyzed and 40 had DIC during the first 24 hours. Clotting times and factors/inhibitors activity used as routine tests failed to identify vascular cells involvement. Compared to normal range, patients with septic shock showed a three-fold increase (p<0.001) in total procoagulant MPs at day (D) 1 that remained elevated at D7. Total circulating microparticle concentrations were in the same range regardless DIC diagnosis in all patients. Different microparticle phenotypes were already detected at admission. Regardless of the presence of DIC (p=0.15), endothelial cell apoptosis was suggested by a major increase in CD31-MPs that remained elevated until D7 (p=0.19). Endothelial activation was assessed by CD105-MPs, which remained dramatically increased in DIC (DIC at D1: 2.7±2.4 nM eq. PhtdSer and DIC at D2: 1.8±1.7 nM eq. PhtdSer, vs. no DIC: 0.8±0.6 nM eq. PhtdSer, p<0.001) and tended to return to baseline at the end of the follow-up period (p=0.05). No alteration in E-selectin (CD62E-MPs) levels could be detected. CD105-MPs (OR 6.55) and CD31-MPs (OR 0.49) were associated with early DIC in multivariate analysis and can originally predict it. Furthermore, leukocyte CD11a-MPs were also higher in DIC highlighting that endothelial cells and leukocytes are first implied in the process of DIC.

Conclusion: Endothelial-derived microparticles are relevant biomarkers of early vascular injury and endothelial dysfunction in septic shock-induced DIC and could prove useful for patients’ stratification.

ANTICOAGULANT PHARMACOLOGICAL MODULATION OF MICROPARTICLE-MEDIATED VASCULAR RESPONSE IN A RAT SEPTIC SHOCK MODEL

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Introduction: Circulating procoagulant microparticles take part in septic shock vascular dysfunction through pro-inflammatory and procoagulant detrimental effects. The objective of this experimental work was to study how circulating procoagulant microparticles are involved in vascular dysfunction of resuscitated septic shock in rats and to assess the effect of microparticle pharmacological modulation by an anticoagulant recombinant human activated protein C treatment.

Methods: In a first set of experiments, microparticles were isolated from sham or septic rats obtained by cecal ligation and puncture, resuscitated and treated by activated protein C. Then, healthy recipient rats were inoculated with an identical amount of microparticles and hemodynamic parameters were recorded during 4 hours. At the end of the record, microparticles and organs were harvested for in-vitro analysis.

Results: Treating septic rats with activated protein C significantly reduces norepinephrine necessary to reach mean arterial pressure goal and reduces the amount of leukocyte-derived microparticles. Microparticles from septic rats significantly decrease mean arterial pressure when infused to healthy recipient rats. Activated protein C prevents this deleterious hemodynamic effect and is associated with elevated microparticle thromboxane content and decreased arterial inflammation. In addition, microparticle phenotype is modulated in recipient rats with increased levels of platelet and endothelial microparticles.

Conclusions: Activated protein C treatment highlighted the possibility to modulate microparticles and showed that they can behave as cellular effectors conveying an anti-inflammatory message resulting in hemodynamic improvement.
DELETION OF SIRT3 DOES NOT AFFECT Atherosclerotic BUT ACCELERATES WEIGHT GAIN AND IMPAIRS RAPID METABOLIC ADAPTATION IN LDL RECEPTOR KNOCKOUT MICE – IMPLICATIONS FOR CARDIOVASCULAR RISK FACTOR DEVELOPMENT

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Introduction: Sirt3 is a mitochondrial NAD+-dependent deacetylase that governs mitochondrial metabolism and reactive oxygen species homeostasis. Sirt3 deficiency has been reported to accelerate the development of the Metabolic Syndrome. However, the role of Sirt3 in atherosclerosis remains enigmatic. We aimed to investigate whether Sirt3 deficiency affects atherosclerotic plaque formation, plaque vulnerability, and metabolic homeostasis.

Methods and Results: Low-density lipoprotein receptor knockout (LDLR−/) and LDLR/Sirt3 double-knockout (Sirt3−/) mice were fed a high-cholesterol diet (1.25% w/w) for 12 weeks. Atherosclerosis was assessed en face in thoracoabdominal aortae and in cross sections of aortic roots. Sirt3 deletion led to hepatic mitochondrial protein hyperacetylation. Unexpectedly, though plasma malondialdehyde levels were increased in Sirt3−/− mice, Sirt3 deletion did not affect plaque burden or features of plaque vulnerability, i.e. fibrous cap thickness and necrotic core diameter. Likewise, plaque macrophage and T cell infiltration as well as endothelial activation, i.e. Vascular Adhesion Molecule-1 expression, remained unaltered. Electron microscopy of aortic walls revealed no difference in mitochondrial micro-architecture between both groups. Interestingly, loss of Sirt3 was associated with accelerated weight gain and an impaired capacity to react to rapid changes in nutrient supply as assessed by indirect calorimetry. Serum lipid levels and glucose tolerance were unaffected by Sirt3 deletion in LDLR−/− mice.

Conclusions: Sirt3 deficiency does not affect advanced atherosclerosis in LDLR−/− mice. However, Sirt3 controls systemic levels of oxidative stress, limits expedited weight gain, and allows rapid metabolic adaptation. Thus, Sirt3 may contribute to postpone cardiovascular risk factor development.

ARGINASE INHIBITION IMPROVES LEFT VENTRICULAR DYSFUNCTION IN MURINE DOxorubicin-INDUCED HEART FAILURE, AND INCREASED ARGINASE ACTIVITY CORRELATES WITH CARDIAC DYSFUNCTION IN PATIENTS WITH SYSTOLIC HEART FAILURE

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Introduction: Imbalance between nitric oxide (NO) and reactive oxygen species (ROS) is important in cardiovascular pathophysiology. Arginase is a pleiotropic enzyme, which not only metabolizes arginine to urea and ornithine, but also regulates NO production by competing with NO synthase for a common substrate arginine. Contrary to the deleterious effect of arginase on endothelial function, its pathophysiological role in heart failure (HF) remains largely unknown. We assessed the hypothesis that arginase inhibition improves left ventricular (LV) systolic dysfunction in murine doxorubicin (Dox)-induced HF, and that increased arginase activity is correlated to the severity in patients with systolic HF.

Method: PBS, Dox (5 mg/kg/week), or Dox plus an arginase inhibitor N-omega-hydroxy-nor-L-arginine (nor-NOHA, 40 mg/day) was administered intraperitoneally to adult male C57BL/6 mice for five weeks. Systolic blood pressure was serially measured by tail-cuff method. Expression and activity of tissue arginase, echocardiography, cardiomyocyte apoptosis, fibrosis, or macrophage accumulation in the LV, and concentration of NO and hydroperoxide in serum and tissues were analyzed at 8 weeks. NO secretion with or without Dox and nor-NOHA from aortic endothelial cells and peritoneal macrophages was examined in vitro. In human study, thirty patients each of chronic systolic HF (age, 69.4 ± 10.2 years; ischemic, 43%; LV ejection fraction (EF), 31.3 ± 10.4%; BNP, 441.7 ± 362.4 pg/ml) and healthy control subjects were registered. We performed echocardiography and brachial-ankle pulse wave velocity, and analyzed plasma concentration of ornithine and arginine and serum concentration of NO, hydroperoxide, and hsCRP. Then, univariate and stepwise multivariate analyses including clinical parameters were performed.

Results: (1) Dox administration to mice increased expression levels and activity of arginase in the lung and liver. (2) Echocardiography revealed that nor-NOHA administration completely reversed Dox-induced decrease in EF, in parallel with expression levels of BNP mRNA, without affecting apoptosis, fibrosis, or macrophage infiltration in the LV. (3) Arginase inhibition reversely lowered systolic blood pressure up to 12.1% only during administration. (4) Importantly, arginase inhibition improved Dox-induced decline in NO concentration and NO/ROS ratio in serum and NO concentration in the aorta and lung, but not in the liver. (5) Furthermore, arginase inhibition stimulated NO secretion from aortic endothelial cells and peritoneal macrophages in vitro. (6) Plasma ornithine/arginine (O/A) ratio, indicating systemic arginase activity, significantly increased in patients with systolic HF compared with controls. Interestingly, O/A ratio was significantly correlated with the inferior vena cava diameter, and a trend was revealed in correlation with BNP level by univariate and stepwise multivariate analyses. O/A ratio was further correlated with pulse wave velocity and decline in serum NO concentration and NO/ROS ratio.

Conclusion: Arginase inhibition improves LV systolic dysfunction in Dox-induced HF in mice. Increase in systemic arginase activity is correlated with preload and afterload of the LV and decline in serum NO concentration in patients with systolic HF. These findings highlight the pathophysiological role of arginase in HF, indicating that arginase may be a novel therapeutic target for vascular complications in the disease.
VASCULAR DYSFUNCTION IN PSORIASIS – A MOUSE EXPERIMENTAL APPROACH

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Introduction: Interleukin-17A (IL-17A) is known to be a central cytokine in psoriasis, an inflammatory skin disease marked by increased cardiovascular mortality. IL-17A has been described to promote vascular dysfunction and has been suspected to link psoriasis and cardiovascular disease but there never has been a mouse experimental approach. We now tried to test if dermal over-production of IL-17A leading to skin inflammation may have indeed systemic side effects by causing arterial hypertension, vascular dysfunction and oxidative stress in psoriasis.

Methods and Results: Mice with a cre-inducible overexpression of IL-17A crossbred with mice harbouring the Cre recombinase under transcriptional control of K14 (K14-IL-17Aind/+ ) were compared with the IL-17Aind/+ controls. The overexpression of IL-17A in the skin resulted in a severe psoriasis-like skin inflammation. Besides, we found an increased cardiovascular reactive oxygen species (ROS) formation (assessed by chemiluminescence and oxidative microtopography), serum levels of IL-17A and IL-6 and circulating CD11b+ inflammatory leukocytes (ELISA and flow cytometry). The psoriasis mice showed an increased systolic blood pressure and compensatory left ventricular hypertrophy and suffered from a vascular dysfunction. In total they had reduced survival compared to age-matched controls (Kaplan-Meyer survival statistics). Immunohistochemistry and flow cytometry revealed in psoriatic mice increased vascular production of the nitric oxide/superoxide reaction product peroxynitrite and infiltration of myeloperoxidase+CD11b+GR1+F4/80+ cells accompanied by increased expression of the inducible NO-Synthase and the NADPH oxidase subunit nox2 (assessed by Western Blot).

Conclusion: We demonstrate that dermal over-expression of IL-17A not only directly promotes psoriatic plaque formation but may be also responsible for the induction of systemic cardiovascular disease.

CLONAL RESTRICTION AND PREDOMINANCE OF REGULATORY T CELLS IN CORONARY THROMBI OF PATIENTS WITH ACUTE CORONARY SYNDROMES

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Aims: Regulatory T cells (Treg) exert prominent anti-inflammatory and atheroprotective effects in experimental atherosclerosis and can be induced against specific antigens using immunization strategies (clonal restriction). No data exist on Treg in combination with clonal restriction of T cells in patients with acute coronary syndromes (ACS).

Methods and Results: Among T cell subsets characterized by flow cytometry, Treg (CD4+Foxp3+) were twice as frequent in coronary thrombi compared with peripheral blood. Genomic DNA was extracted from coronary thrombi and peripheral blood in order to evaluate T cell receptor β chain diversity by means of Multi-N-plex PCR using a primer specific for all T cell receptor β V gene segments and a primer specific for T cell receptor β J gene segments. T cell receptor diversity was reduced in thrombi compared with peripheral blood (intra-individual comparisons in 16 patients) with 8 gene rearrangements in the T cell receptor common in at least 6 out of 16 analyzed coronary thrombi. Compared with age-matched healthy probands (n=16), T cell receptor diversity was also reduced in peripheral blood of patients with ACS; these findings were independent of peripheral T cell numbers.

Conclusion: We provide novel evidence for a severely perturbed T cell compartment characterized by clonal restriction in peripheral blood and coronary thrombi from patients with ACS. Treg prevailed among T cell subsets identified in coronary thrombi, suggesting Treg as a novel target for specific therapies such as immunization aimed at boosting the anti-inflammatory component of adaptive immunity in coronary atherothrombosis.
ARGINASE II PROMOTES PRE-ADIPOCYTE IL-6 PRODUCTION LEADING TO ENDOTHELIAL INFLAMMATION

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Introduction: Both adipose tissue macrophages (ATMs) and adipose tissue stromal cells (ATSCs) including preadipocytes and fibroblasts are implicated in adipose inflammation that is mechanistically linked with chronic inflammatory diseases including type-II diabetes and atherosclerosis. Our previous studies demonstrated that arginase-II (Arg-II) promotes endothelial dysfunction and macrophage inflammation contributing to insulin resistance and atherosclerosis. Here we investigate the role and mechanism of Arg-II in inducing inflammation of 3T3-L1 preadipocytes and its interaction with endothelial inflammation.

Methods: Overexpression of Arg-II in 3T3-L1 preadipocytes (from ATCC) was mediated by transducing recombinant adenovirus. The effect of Arg-II on inflammation was assessed by monitoring the expression and secretion of cytokines including IL-6, TNF-α and MCP-1 by quantitative real time PCR (qRT-PCR) and ELISA, respectively. The conditioned medium of 3T3-L1 cells under different experimental condition was used to investigate the ATSC’s effects on endothelial inflammation.

Results: Overexpressing Arg-II in 3T3-L1 cells upregulates IL-6 level, but not TNFα or MCP-1, at both mRNA and secreted protein levels. Moreover, overexpressing Arg-II induces p38mapk signalling pathway in 3T3-L1 cells, which reaches the highest level at 60h and 84h post-transduction. Inhibition of p38mapk with the specific p38mapk inhibitor SB203580 significantly reduces Arg-II-induced IL-6 production in 3T3-L1 cells. Furthermore, conditioned medium of 3T3-L1 cells overexpressing Arg-II enhances vascular adhesion molecule-1 (VCAM-1) but not intercellular adhesion molecule-1 (ICAM-1) in human endothelial cells, which can be partially inhibited by neutralizing antibody against IL-6.

Conclusions: Arg-II promotes IL-6 expression and secretion in pre-adipocytes through activation of p38mapk, leading to enhanced adhesion molecule expression in endothelial cells, which may contribute to vascular disease associated with obesity.

COMPARISON OF LINAGLITIN, SITAGLITIN AND LIRAGLUTIDE EFFECTS ON SURVIVAL AND VASCULAR COMPLICATIONS IN EXPERIMENTAL SEPSIS

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Introduction: Gliptins (dipeptidyl peptidase [DPP]-4 inhibitors) are a new class of drug for the treatment of hyperglycemia and recent studies revealed anti-inflammatory effects of DPP-4 inhibitors in experimental atherosclerosis and septic animals. The aim of the present study was to compare the effect of linagliptin with an alternative DPP-4 inhibitor, sitagliptin, and the direct glucagon-like peptide (GLP)-1 analogue liraglutide on survival and vascular complications in different experimental models of septic shock.

Methods: Mice or rats were treated with linagliptin, sitagliptin or liraglutide for 4-7 days. On Day 3 or 6 of treatment, mice or rats were injected with lipopolysaccharide (LPS, 10, 17.5 or 20 mg/kg i.p.) to induce septic shock. DPP-4-/- mice served as an additional control group. Survival was monitored over time and vascular function, nitrosyl-iron hemoglobin in whole blood or oxidative stress were measured 24 h after LPS treatment. The Gehan-Breslow-Wilcoxon-Test was used for statistical analysis of Kaplan-Meier curves.

Results: Linagliptin and liraglutide therapy or DPP-4 deficiency improved the survival of septic mice. Liraglutide improved vascular dysfunction in septic rats without improvement of inflammatory parameters such as nitrosyl-iron hemoglobin in whole blood or leukocyte-dependent oxidative stress. Linagliptin and to a minor extend sitagliptin improved vascular dysfunction in septic rats with significant suppression of inflammatory parameters.

Conclusion: The therapy of diabetic patients with linagliptin and liraglutide is well established. The results reported here on the improvement in the survival of septic animals could provide additional evidence for the potential use of these drugs in patients with septic shock.
NOVEL SIRT1 ACTIVATOR SRT3025 PROVIDES ATHEROPROTECTION THROUGH INCREASED HEPATIC EXPRESSION OF LOW-DENSITY LIPOPROTEIN RECEPTOR

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Introduction: Increased plasma low-density lipoprotein (LDL) cholesterol is a driving force of atherosclerosis. Hepatic LDL receptor (LDL-R) expression confers atheroprotection by removing cholesterol from the blood stream. Genetic studies of SIRT1 have predominantly shown to have beneficial effects in metabolic diseases. However, its role in atherosclerosis and lipid metabolism remains controversial. We compared the effects of pharmacological SIRT1 activation on lipid metabolism and atherosclerosis in apolipoprotein E knockout (ApoE−/−) and LDL-R knockout (LDL-R−/−) mice.

Methods: We placed male ApoE−/− or LDL-R−/− mice on a high-cholesterol diet (1.25% w/w) or the same diet supplemented with the SIRT1 activator SRT3025 (3.18g/kg diet; Sirtris, Cambridge, MA, USA) for 12 weeks and then assessed plasma lipids and cytokines, severity of aortic atherosclerosis, hepatic lipid content and hepatic expression of genes involved in inflammation and lipid homeostasis. In addition, we assessed the effects of SRT3025 on LDL-R expression in hepatic AML12 cells in vitro.

Results: ApoE−/− mice treated with SRT3025 showed less atherosclerosis and steato-hepatitis and had lower levels of plasma cholesterol (LDL- and total cholesterol), TNFα, MCP-1, and IL-6 compared with placebo-treated control mice. LDL-R expression was increased 10-fold in drug-treated ApoE−/− mice. Corresponding SRT3025 experiments in LDL-R−/− mice revealed a loss of atheroprotection without a decrease in plasma cholesterol. In vitro SIRT1 activator treatment of hepatic AML12 cells induced LDL-R expression; this increase was abolished after SIRT1 knockdown.

Conclusions: Our findings identify induction of hepatic LDL-R expression as a novel downstream effect of SIRT1 activity and highlight the potential of SIRT1 activation as an anti-atherosclerotic strategy.

REPROGRAMMING OF AGING AND LONGEVITY GENES p66Shc AND JUND BLUNTS AGE-RELATED DYSFUNCTION OF ANGIOGENIC EARLY OUTGROWTH CELLS

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Introduction: Cardiovascular disease is markedly age-dependent. Early outgrowth cells (EOCs) are important modulators of the vascular repair process, favouring myocardial neovascularization. Impairment of EOCs functionality in human aging is mostly driven by reactive oxygen species (ROS), but the molecular mechanisms remain largely unknown. We previously reported that transcription factor JunD and mitochondrial adaptor p66Shc are critically involved ROS-induced vascular aging. The present study investigates the role of JunD and p66Shc in age-related EOCs dysfunction.

Methods: EOCs were isolated and cultured from peripheral blood mononuclear cells of young (24±4 years; n=5) and old (63±5 years; n=6) healthy volunteers enrolled via the blood donation service of the University Hospital Zürich, Switzerland. Gene silencing of p66Shc was performed with siRNA technology (Microsynth®), while JunD overexpression was obtained with a predesigned vector (Origene®). Scrambled siRNA or empty vector were used as negative controls for p66Shc and JunD, respectively. Three days after transfection young and old EOCs were harvested for measurement of O2− levels by ESR spectroscopy, migration assay and real-time PCR. Written informed consent was obtained from all subjects.

Results: EOCs isolated form old individuals showed higher p66Shc expression and JunD downregulation as compared with young subjects. p66Shc and JunD deregulation in old EOCs was associated with increased O2− generation, blunted migration, upregulation of the NADPH subunit Nox2 as well as reduced expression of the scavenger enzymes manganese superoxide dismutase (MnSOD) and aldehyde dehydrogenase-2 (ALDH2). Interestingly, either p66Shc knockdown or JunD overexpression significantly suppressed age-related O2− production, improved EOCs migration and restored the balance between oxidant and antioxidant enzymes.

Conclusions: p66Shc and JunD are critically involved in age-dependent EOCs dysfunction by altering their redox state. Modulation of such aging and longevity genes restores normal repair capacities in EOCs from aged individuals and may be useful as an ex vivo strategy to improve the clinical efficacy of stem cell therapy in elderly cardiovascular patients.
PLEIOTROPIC EFFECTS OF TICAGRELOR BUT NOT CLOPIDOGREL ON HUMAN ENDOTHELIAL CELLS

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Background: Platelet inhibitors targeting P2Y12 receptor (clopidogrel, prasugrel and ticagrelor) play a major role in the treatment of acute coronary syndrome (ACS) and in secondary prevention of cardio- and cerebrovascular disease. Different P2Y12 receptor antagonists have shown diverse beneficial effects in clinical trials; particularly, ticagrelor was shown to reduce mortality due to vascular causes, myocardial infarction and stroke in PLATO. However, these effects may not necessarily be platelet-dependent since P2Y12 receptors are not only found on platelets; additionally, part of the observed effects may also result from P2Y12-independent responses.

Methods: To investigate the role of P2Y12 receptor antagonists on endothelial dysfunction, primary human aortic endothelial cells (HAECs) stimulated with or without TNFa (10 ng/ml) were treated with increasing concentrations of clopidogrel active metabolite (CAM) (1.5 x 10^{-8}M, 1.5 x 10^{-7}M, 1.5 x 10^{-6}M) or ticagrelor (10^{-7}M, 10^{-6}M and 10^{-5}M). Protein levels of endothelial mediators (eNOS, COX-1, COX-2, VCAM-1 and ICAM-1) were determined by western blotting. To confirm the expression of P2Y12 receptor in human aortic, brain and cardiac endothelial cells at the protein and mRNA level, western blotting and qRT-PCR were performed.

Results: Ticagrelor, unlike CAM induced a dose-dependent phosphorylation of eNOS at serine 1177 in TNFa-stimulated HAECs vs TNFa alone (TNFa: 100 %; TNFa+tica 10^{-7}M: +178±45%, n=8, p=ns; TNFa+tica 10^{-6}M: +196±36%, n=8, p<0.05; TNFa+tica 10^{-5}M: +254.1±44%, n=8, p<0.005). TNFa induced VCAM-1 expression in endothelial cells, which was decreased by ticagrelor at the highest concentration tested (TNFa: 100 %; TNFa+tica 10^{-5}M: 73±11%, n=8, p<0.05). However, ICAM-1 expression was not affected by CAM or ticagrelor. Furthermore, ticagrelor but not CAM induced COX-2 expression in TNFa-stimulated cells (TNFa: 100 %; TNFa+tica 10^{-5}M: +445±97%, n=8, p<0.005), while no changes in COX-1 expression were observed. Surprisingly, P2Y12 receptor expression was not detected in primary human aortic endothelial cells of aortic, cerebral or cardiac origin, neither at the protein nor at the mRNA level.

Conclusions: Ticagrelor treatment in human TNFa-stimulated aortic endothelial cells results in 1) increased activation of eNOS by phosphorylation at serine 1177, 2) reduced expression of VCAM-1, and 3) increased expression of COX-2. Thus, ticagrelor, but not CAM demonstrates platelet-independent effects, by directly affecting endothelial cells. Additionally, in view of the undetected expression of P2Y12, the observed effect may also be P2Y12 receptor independent. The physiological relevance of our data will be further investigated in vivo.

THE ADAPTOR PROTEIN P66SHC MEDIATES PATHOLOGICAL CYCLIC STRETCH-INDUCED ENDOTHELIAL DAMAGE

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Introduction: Increased cyclic stretch to the vessel wall, as observed in hypertension, leads to endothelial dysfunction through increased reactive oxygen species (ROS) production and reduced nitric oxide (NO) bioavailability. Genetic deletion of the adaptor protein p66shc protects mice against age-related and hyperglycemia-induced endothelial dysfunction, atherosclerosis and stroke. Since the role of p66shc in mediating mechanical forces-induced O2- production and decreased NO bioavailability is unknown, we studied the effect of cyclic stretch on p66shc in endothelial cells (EC) in vitro and in aortas of hypertensive rats in vivo.

Methods and Results: Primary human aortic EC were exposed to cyclic stretch. p66shc phosphorylation at Ser36 was increased in a stretch-, and time-dependent manner. Pathological stretch increased O2- generation and reduced NO bioavailability. Silencing of p66shc blunted pathological stretch-induced O2- generation and restored NO bioavailability downstream of c-Jun N-terminal kinase (JNK1/2) and integrin alpha5beta1. Moreover, spontaneously hypertensive rats showed increased aortic p66shc protein levels and an increased activation of p66shc.

Conclusions: Thus, stretch by activating integrin alpha5beta1 and JNK1/2 phosphorylates p66shc at Ser36, initiates O2- production and in turn causes endothelial dysfunction due to a reduced NO bioavailability both in human EC in vitro as well as in aortas of hypertensive rats in vivo. This novel molecular pathway may be crucial for endothelial dysfunction and vascular disease in hypertension.
MOLECULAR MECHANISMS OF THE CROSSTALK BETWEEN MITOCHONDRIA AND NADPH OXIDASE THROUGH REACTIVE OXYGEN SPECIES IN WHITE BLOOD CELLS – IMPLICATIONS FOR CARDIOVASCULAR DISEASES

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Aims: Oxidative stress is involved in the development of cardiovascular disease. There is growing body of evidence for a crosstalk between different enzymatic sources of oxidative stress. With the present study we sought to determine the underlying crosstalk mechanisms, the role of the mitochondrial permeability transition pore (mPTP) and its link to endothelial dysfunction. The high clinical impact of this crosstalk is obvious considering a clinical trial of Piot et al. (2008, NEJM). In this study the blockade of mPTP by Cyclosporin A showed cardio protective abilities on patients with myocardial infarction.

Results: NADPH oxidase (Nox) activation and triggered oxidative burst was assessed by chemiluminescence or fluorescence-based assays and translocation of cytosolic Nox subunits in response to mitochondrial reactive oxygen species (mtROS) formation in human leukocytes. mtROS induced Nox activation was prevented by inhibitors of the mPTP, protein kinase C, tyrosine kinase cSrc or Nox activity and an intracellular calcium chelator. mtROS induced Nox-dependent oxidative burst was absent in white blood cells with p47phox deficiency (regulates Nox2) or with cyclophilin D deficiency (regulates mPTP), whereas this crosstalk was amplified in white blood cells with mitochondrial manganese superoxide dismutase deficiency (MnSOD+/-).

Increases in blood pressure, endothelial dysfunction, endothelial nitric oxide synthase dysregulation/uncoupling or Nox activity in response to angiotensin II in vivo treatment were more pronounced as compared to untreated controls, which was further aggravated in MnSOD+/- mice and improved by cyclophilin D deficiency.

Conclusions: Our data show that mtROS trigger the activation of phagocytic and cardiovascular NADPH oxidases, which may have fundamental consequences on immune cell activation and progression of angiotensin II-mediated hypertension.
ENDOTHELIAL-DERIVED MICROPARTICLES CONTRIBUTE TO DISSEMINATED INTRAVASCULAR COAGULOPATHY DURING SEPTIC SHOCK

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Introduction: Septic shock-induced disseminated intravascular coagulopathy (DIC) contributes to endothelial dysfunction, multiple organ failure and high mortality rate. The objective of this study was to assess the potential role of procoagulant microparticles (MPs) as time course strong biomarkers of vascular cells contribution to DIC.

Patients and methods: Prospective multicenter study on one hundred consecutive adult patients with septic shock enrolled at admission in medical intensive care units. Hemostasis parameters were analyzed and microparticles measured by pro thrombinase assay after capture onto insolubilized annexin-5 or biotynilated antibodies to determine their cell origin.

Results: 92 patients were analyzed and 40 had DIC during the first 24 hours. Clotting times and factors/inhibitors activity used as routine tests failed to identify vascular cells involvement. Compared to normal range, patients with septic shock showed a three-fold increase (p<0.001) in total procoagulant MPs at day (D) 1 that remained elevated at D7. Total circulating microparticle concentrations were in the same range regardless DIC diagnosis in all patients. Different microparticle phenotypes were already detected at admission. Regardless of the presence of DIC (p=0.15), endothelial cell apoptosis was suggested by a major increase in CD31-MPs that remained elevated until D7 (p=0.19). Endothelial activation was assessed by CD105-MPs, which remained dramatically increased in DIC (DIC at D1: 2.7±2.4 nM eq. PhtdSer and DIC at D7: 2.8±1.7 nM eq. PhtdSer, vs. no DIC: 0.8±0.6 nM eq. PhtdSer, p=0.001) and tended to return to baseline at the end of the follow-up period (p=0.05). No alteration in E-selectin (CD62E-MPs) levels could be detected. CD105-MPs (OR 6.55) and CD31-MPs (OR 0.49) were associated with early DIC in multivariate analysis and can originally predict it. Furthermore, leukocyte CD11a-MPs were also higher in DIC highlighting that endothelial cells and leukocytes are first implied in the process of DIC.

Conclusion: Endothelial-derived microparticles are relevant biomarkers of early vascular injury and endothelial dysfunction in septic shock-induced DIC and could prove useful for patients’ stratification.

HERC2 AS AN E3 LIGASE MEDIATING SIRT1-INDUCED LKB1 DEGRADATION IN ENDOTHELIAL CELLS: IMPLICATION IN ENDOTHELIAL SENESCENCE

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Introduction: During vascular aging, endothelial cells become senescent and exhibit decreased capacity of regeneration, which facilitates the development of atherosclerotic cardiovascular diseases. In senescent endothelial cells, a protein deacetylase and longevity regulator SIRT1 is down-regulated, whereas its protein target LKB1, a serine/threonine kinase, is up-regulated. Increasing the expression and activity of SIRT1 blocks cellular senescence and enhances endothelial regeneration, in part by promoting ubiquitination and protein degradation of LKB1. HERC2 (HECT domain and RLD 2) is a giant protein that has been implicated in regulating genome stability, cell cycle progression and circadian clock controls. It contains an E3 ubiquitin ligase domain. The present study investigated the role of HERC2 in SIRT1-mediated LKB1 degradation during the development of endothelial senescence.

Methods and Results: Prolonged culture of porcine aortic endothelial cells (PAECs) led to replicative senescence and a decreased expression of HERC2. Knocking down HERC2 with specific siRNA induced cellular senescence in PAECs, in conjunction with an augmented LKB1 protein expression. Co-immunoprecipitation revealed that HERC2 interacted with both SIRT1 and LKB1. Over-expression of SIRT1 promoted the interactions between HERC2 and LKB1. In vitro ubiquitination experiment confirmed that HERC2 acted as an E3 ligase that targeted LKB1 for degradation. Ubiquitination of LKB1 was significantly reduced in senescent PAECs with reduced HERC2 expression. SIRT1 facilitated HERC2-mediated LKB1 ubiquitination. In both PAECs and mice arteries, the circadian oscillation of HERC2 was similar to SIRT1, but inversely correlated with that of LKB1.

Conclusion: HERC2 prevents endothelial senescence by acting as an E3 ligase to decrease LKB1 protein stability, which contributes to the anti-endothelial senescence and anti-vascular aging activities of SIRT1.
ARGINASE-II INDUCES ENDOTHELIAL SENESCENCE-ASSOCIATED SECRETORY PHENOTYPE THROUGH P38MAPK

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Introduction: p38mapk has been shown to regulate cellular senescence-associated secretory phenotype (SASP) characterized by secretion of numerous growth factors, cytokines, chemokines, matrix metalloproteinases (MMPs) and other proteins in senescent cells. Our previous work demonstrated that augmented arginase-II (Arg-II) expression/activity promotes endothelial inflammation and cell senescence through uncoupling of endothelial nitric oxide synthase (eNOS). The aim of this study is to investigate whether Arg-II induces eNOS uncoupling as well as SASP through p38mapk.

Method: Human endothelial cells were isolated from umbilical veins (HUVECs). Young and replicative senescent HUVECs were prepared for in vitro study. Superoxide anion and NO productions were measured by DHE and DAF-2DA staining, respectively. Secreted cytokines including Interlukin-6 (IL-6), Interlukin-8 (IL-8), and monocyte chemotactic protein-1 (MCP-1) in the conditioned medium from endothelial cells were measured by ELISA. p38mapk phosphorylation was detected by immunoblotting. The role of Arg-II and p38mapk in SASP were examined by silencing these genes in senescent HUVECs or treating the senescent cells with p38mapk inhibitor, and by overexpressing the Arg-II in young HUVECs.

Results: Senescent endothelial cells show increased IL-6, IL-8, and MCP-1 expression/secretion and enhanced p38mapk activation with concurrent elevated Arg-II level as compared to the young cells, indicating that p38mapk may be involved in Arg-II-induced SASP. Silencing Arg-II or p38mapk-alpha, or inhibition of p38mapk with inhibitor SB203580 in senescent cells decreases the pro-inflammatory cytokine secretion and recouples eNOS function. Moreover, overexpression of Arg-II gene in young endothelial cells activates p38mapk and increases secretion of the pro-inflammatory cytokines; Inhibition of p38mapk blunted Arg-II-induced endothelial cytokine production under this condition.

Conclusion: Arg-II promotes endothelial SASP through activation of p38mapk. These results suggest that both Arg-II and p38mapk may provide a novel therapeutic target for vascular aging and related cardiovascular diseases.

ARGINASE-II INDUCES VASCULAR SMOOTH MUSCLE CELL SENESCENCE AND APOPTOSIS THROUGH P66SHC AND P53 INDEPENDENTLY OF ITS ENZYMATIC ACTIVITY: IMPORTANCE IN ATHEROSCLEROTIC PLAQUE VULNERABILITY

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Introduction: Vascular smooth muscle cell (VSMC) senescence and apoptosis are involved in atherosclerotic plaque vulnerability. Arginase-II (Arg-II) has been shown to promote endothelial dysfunction and plaque vulnerability phenotypes in mice through uncoupling of endothelial nitric oxide synthase (eNOS) and activation of macrophage inflammation. The function of Arg-II in VSMC with respect to plaque vulnerability is unknown. This study investigated functions of Arg-II in VSMC linking to plaque vulnerability.

Method: VSMCs were isolated from human umbilical veins. Non-senescent and replicative senescent cells were prepared. Overexpression or silence of the targeting gene in VSMCs was mediated by transducing recombinant adenovirus. 10 weeks old male Apo E^-/- Arg-II^+/+ and Apo E^-/- Arg-II^-/- mice fed a high fat diet for 10 weeks were used to investigate the role of Arg-II in VSMC apoptosis/senescence linking to plaque vulnerability in the atherosclerosis animal model.

Results: In non-senescent VSMC, overexpression of wild type Arg-II or an enzymatically inactive Arg-II mutant (H160F) causes similar effects on mitochondrial dysfunction, cell apoptosis and senescence, which are abrogated by silencing p66Shc or p53. The activation of p66Shc but not p53 by Arg-II is dependent on 40S ribosomal protein S6 kinase 1 (S6K1). In senescent VSMC, levels of Arg-II, S6K1-p66Shc-, and p53-signalings are increased, which are inhibited by silencing Arg-II, resulting in reduced cell senescence/apoptosis. Conversely, silencing p66Shc in the senescent cells is also able to reduce S6K1-signaling and Arg-II levels, demonstrating a positive crosstalk among Arg-II, S6K1, and p66Shc. Furthermore, genetic ablation of Arg-II in the atherosclerosis-prone apolipoprotein E-deficient (Apo E^-/-) mice reduces the aforementioned signalings and apoptotic VSMC in the plaque of aortic roots.

Conclusion: Arg-II, independently of its L-arginine ureahydrolase activity, promotes mitochondrial dysfunction leading to VSMC senescence/apoptosis through a complex positive crosstalk among S6K1, p66Shc and p53, contributing to atherosclerotic vulnerability phenotypes in mice.
ARGINASE-II SUPPRESSES ENDOTHELIAL AUTOPHAGY THROUGH S6K1 AND P53 INDEPENDENTLY OF ITS L-ARGININE UREAHYDROLASE ACTIVITY: IMPLICATION IN ATHEROSCLEROTIC PLAQUE VULNERABILITY

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Introduction: Autophagy is a process of cell self-cannibalization by which cytoplasmic components are degraded to remove dysfunctional organelles/proteins and to generate energy-rich compounds. It is predominantly a cytoprotective rather than a self-destructive process and contributes to cellular recovery in an unfavorable environment. Reduced autophagy has been associated with accelerated aging and aging-associated diseases including atherosclerosis, whereas increased autophagy delays aging and stabilizes atherosclerotic plaques. Our previous study demonstrated that enhanced arginase-II (Arg-II) promotes endothelial aging through endothelial nitric oxide synthase (eNOS) uncoupling and genetic ablation of Arg-II decelerates vascular aging and confers plaque more stable in mice. Here, we investigate the role of Arg-II in vascular endothelial autophagy with respect to aging and atherosclerotic plaque vulnerability.

Method: Human endothelial cells were isolated from human umbilical veins (HUVECs). Young and replicative senescent HUVECs were prepared for in vitro experiments. Male Apo E^-/- Arg-II^+/+ and Apo E^-/- Arg^-II-/- mice fed a high fat diet for 10 weeks were employed as an animal model related to aging and atherosclerotic plaques vulnerability. Expression level of Light Chain 3-II (LC3-II) was used as marker for autophagy by immunoblotting or immunostaining.

Results: In young HUVECs, overexpression of wild type Arg-II or an enzymatically inactive Arg-II mutant (H160F) significantly suppresses the expression level of LC3-II, which is prevented by silencing 40S ribosomal protein S6 kinase 1(S6K1) or tumor suppressor protein p53, both of which are activated upon overexpression of Arg-II or H160 as monitored by enhanced phosphorylation of S6 ribosomal protein (S6) at Serine 235/236 and p53 at Serine 15, respectively. On the contrary, AMP-activated protein kinase (AMPK), an autophagy inducer, is inhibited by Arg-II or H160F. Moreover, when compared to young HUVECs, replicative senescent HUVECs display inferior autophagy capability. Silencing Arg-II, S6K1 or p53 in senescent HUVECs prevents aging-associated autophagy inhibition. In addition, genetic ablation of Arg-II in the atherosclerosis-prone apolipoprotein E-deficient (Apo E^-/-) mice strongly enhances autophagy as revealed by enhanced LC3-II levels in the plaque of aortic roots.

Conclusion: Arg-II blunts endothelial autophagy through activation of S6K1 and p53 with concomitant inhibition of AMPK independently of its L-arginine ureahydrolase activity, contributing to vulnerable plaque formation. Targeting Arg-II may provide a promising therapeutic strategy for stabilizing plaques through induction of autophagy.
ANODAL IONTOPHORESIS OF A SOLUBLE GUANYLYL CYCLASE STIMULATOR INDUCES A SUSTAINED INCREASE IN SKIN BLOOD FLOW IN RATS

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Introduction: The treatment of systemic sclerosis-related digital ulcers is challenging. While the only effective drugs are prostacyclin analogues, their use is limited by vasodilation-related adverse reactions. In this study we assessed the local iontophoresis administration of three soluble guanylate cyclase (A-350619, SIN-1 and CFM 1571) and two non-prostanoid IP agonists (MRE-269 and BMY 45778) to induce vasodilation onto the hindquarters of anaesthetized rats.

Methods: Skin blood flow was quantified using laser Doppler imaging during the whole experience, tolerance was assessed by continuously recording blood pressure and histopathologic examination.

Results: Anodal iontophoresis of A-350619 (7.54 mM) induced a sustained increase in cutaneous blood flow (P=0.008 vs control). All other drugs exhibited poor or no effect on skin blood flow. Vasodilation with A-350619 iontophoresis was concentration dependent (7.5 mM, 0.75 mM and 0.075 mM; P<0.001, Jonckheere-Terpstra trend test. This study also compared continuous vs intermittent iontophoresis protocols.

Conclusion: Continuous, anodal iontophoresis of A-350619 at 7.5 mM, increases cutaneous blood flow with good local tolerance. Iontophoresis of sGC stimulators should be investigated as potential local therapy for digital ulceration in patients with scleroderma.

PHOSPHODIESTERASE-5 INHIBITORS FOR THE TREATMENT OF SECONDARY RAYNAUD’S PHENOMENON: SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED TRIALS

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Introduction: Recent controlled trials have assessed the efficacy of PDE-5 inhibitors in secondary Raynaud’s Phenomenon (RP). However, the conclusions are conflicting and whether these drugs are effective remains unclear. The objective of this meta-analysis was to determine the efficacy of PDE-5 inhibitors on the Raynaud’s Condition score (RCS), the frequency and the duration of attacks.

Methods: A systematic review of articles was performed (sources included Medline, Embase, Web of Science, the Cochrane Central Register of Controlled Trials). Only double-blind, randomized, controlled trials (RCTs) were included. Study selection was done independently by 2 authors using predefined data fields, including study quality indicators.

Results: Six RCTs were included (1 with sildenafil, 1 with modified-release sildenafil, 3 with tadalafil and 1 with vardenafil). PDE-5 inhibitors significantly decrease mean RCS by -0.46 [-0.74; -0.17] (p=0.002), the daily frequency of ischaemic attacks by -0.49 [-0.71; -0.28] (p<0.0001), and daily duration of RP attacks by -14.62 [-20.25; -9] min (p<0.0001).

Conclusion: PDE-5 inhibitors appear to have a significant but moderate efficacy in secondary RP
EIGHT WEEKS HOME-BASED HIGH FREQUENCY EXERCISE TRAINING IS NOT SUFFICIENT TO AFFECT VASOACTIVE PEPTIDES IN PATIENTS WITH STABLE CORONARY ARTERY DISEASE.

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Introduction: Exercise is known to have positive effects on endothelial function and also affects clinical outcomes in patients with coronary artery disease (CAD). Endothelin (ET-1) is primarily a vasoconstrictive peptide produced in endothelial cells. Exercise training has been shown to reduce ET-1 in patients with impaired glucose tolerance, pre-hypertension and hypertension. Patients with CAD have severe endothelial dysfunction and higher amounts of ET-1. Relaxin, which is a potent vasodilator, inhibits ET-1 and also has antifibrotic properties. We hypothesized that 8 weeks of home-based high frequency exercise (HFE) in patients with stable CAD would decrease circulating ET-1 levels, and possibly induce Relaxin-2.

Method: Patients with angiographically verified, stable CAD were randomized to HFE for 8 weeks or usual lifestyle. The exercise program consisted of aerobic exercise on a bicycle ergometer 30 min, five days a week at 60-80% of VO2max. Peripheral blood was collected and plasma was stored in -80°C. Plasma ET-1 and Relaxin-2 were analyzed using quantitative sandwich enzyme-linked immunosorbent assay.

Results: Data are presented as median ± standard deviation. There was no difference in ET-1 relative change (baseline and 8 weeks) between controls (n=13) and HFE (n=14), -2.92±34 vs 8.0±38 %, p=NS. Also no difference in Relaxin-2 change could be seen between controls and HFE (2.85±11 vs 4.46±31%), p=NS. No significant changes could be seen between baseline or 8 weeks ET-1 or relaxin-2 concentrations between the two groups.

Conclusion: Although exercise of corresponding duration and intensity is known to decrease ET-1 in other patient groups, this exercise is not sufficient to result in those effects in patients with stable CAD. Patients with CAD are expected to have severe endothelial dysfunction, which might require exercise of longer duration or a more intense exercise program to affect these vasoactive peptides.
SKIN IONTOPHORESIS OF TREPROSTINIL, A PROSTACYCLIN ANALOGUE, INDUCES A SUSTAINED DIGITAL VASODILATION IN HEALTHY SUBJECTS AND PATIENTS WITH SCLERODERMA

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Introduction: Systemic sclerosis (SSc) is a rare disease affecting the skin microcirculation. Digital ulcers are a complication of SSc-related vasculopathy associated with significant morbidity. Therapy of SSc-related ulcerations remains challenging. We previously showed that topical administration of treprostinil, a prostacyclin analogue, through iontophoresis induces a sustained vasodilation in rats (Blaise et al. Br J Pharmacol 2011) and on the forearm of healthy subjects (Blaise et al. J Clin Pharmacol 2013). The objectives of the present work were 1/ to assess intradermal vs plasma concentrations of iontophoretically-administered treprostinil, and 2/ to assess the effect of different protocols of treprostinil iontophoresis on the finger pad of healthy subjects and patients with scleroderma.

Methods: Twenty-two healthy volunteers were recruited. In a first study twelve participants simultaneously received cathodal iontophoresis of treprostinil and NaCl on the right forearm. Two microdialysis fibers were inserted at the dermis-hypodermis junction under the treprostinil site, and samples were collected every hour during 8h. Eight blood samples were taken on the contralateral forearm. The second study aimed at comparing the effect of three doses on digital skin blood flux (quantified using laser speckle contrast imaging). Blood pressure was recorded continuously during all experiments and data were expressed as cutaneous vascular conductance (CVC). In the third study, this protocol was tested in patients with systemic sclerosis.

Results: Iontophoresis of treprostinil 250 μM induced a sustained increase in cutaneous CVC on the forearm (p=0.02 vs NaCl). Intradermal concentration of treprostinil was maximal at H2 and below the detection threshold in all but two participants at H8, whereas plasma concentrations were <1.7 pg/mL at all points for all subjects. Among the three doses tested on the finger pad, iontophoresis at 240 mC/cm² induced a significant increase in CVC (p=0.01, figure 1). There was no local or systemic adverse event. In patients with SSc, cathodal iontophoresis of treprostinil, but not NaCl, increased skin blood flow on the finger pad.

Conclusion: Iontophoresis allowed non-invasive administration of treprostinil 250 μM into the dermis, without systemic diffusion of the drug. Digital cathodal iontophoresis at 240 mC/cm² significantly increased skin blood flow on the finger pad in healthy volunteers and in patients with systemic sclerosis. Cathodal iontophoresis of treprostinil may be a way of getting around the systemic side effects of prostacyclin analogues in patients with digital ulcerations.

Figure 1. Skin vasodilation observed following cutaneous iontophoresis of treprostinil 250 μM at 240 mC/cm² on the index finger pad and placebo on the middle finger pad. Skin blood flow was assessed using Laser Speckle Contrast Imaging.
RAPTOR DEFICIENCY IN ENDOTHELIUM AMPLIFIES CYCLOOXYGENASE-DEPENDENT ENDOTHELIUM-DERIVED CONTRACTIONS

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Introduction: Rapamycin (sirolimus) has a potent anti-proliferative capacity through inhibition of the cell growth controller mammalian target of rapamycin complex 1 (mTORC1). Therefore, sirolimus-eluting stents (SES) are implanted during percutaneous coronary angioplasty and they remarkably inhibit in-stent neo-intimal hyperplasia. Systemic adverse effects appear to be low; however, adverse effects on local endothelium-dependent vasomotor responses distal to the SES implantation segment have been demonstrated in patients and in porcine experimental models. The effects of rapamycin on vasomotion can hypothetically be caused by a direct effect on the endothelium, on the signaling pathway of the endothelium to the medial smooth muscle cells or a direct effect on the media. Here we tested whether endothelial mTORC1 deficiency leads to vascular dysfunction in mouse carotid artery.

Method: The essential regulatory protein raptor that is necessary to assemble a functional mTOR complex 1 was knocked out using a tamoxifen-inducible and endothelium-specific Cre-recombinase approach in 4 week old mice (raptor ec-/-). Reactivity of carotid conduit vessels was assessed ex vivo when mice were 10 month old.

Results: Carotid rings from raptor ec-/- mice displayed 1.9-fold higher maximal contractions as compared with rings from control mice after treatment with vasoconstrictor phenylephrine (300 nM, n=7). This increase in contraction was due to decreased nitric oxide availability as inhibition of nitric oxide synthase (NOS) by L-NAME (100 μM) increased contractions of carotids from control mice to levels observed for raptor ec-/- carotids (n=7). Furthermore, increased contraction of raptor ec-/- carotids was blunted by treatment with cyclooxygenase (COX) inhibitor meclofenamate (1 μM, n=4). Acetylcholine (ACh)-induced relaxation was similar in carotid rings from both groups of mice. However, in raptor ec-/- carotid rings, endothelium-dependent contractions were raised markedly at ACh concentrations of 1 μM (1.7-fold; n=6-7).

Conclusion: Thus, functional mTORC1 in the endothelium is essential to maintain normal vascular function in conduit arteries, particularly by inhibiting cyclooxygenase-dependent vascular contractions. We therefore propose that abnormal vasomotor response in patients with SES originate from a direct cyclooxygenase-dependent effect of mTORC1 inhibition by rapamycin in the endothelium.

ROLE OF ADHERENT PLATELETS IN 12-LIPOOXYGENASE-MEDIATED VASCULAR RELAXATION IN MOUSE ARTERIES

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Lipoxygenase (LO) metabolites of arachidonic acid (AA) regulate vascular tone in mice. Previously, vasoactive LO metabolites such as 11,12,15-trihydroxy-eicosatrienoic acid or 12(S)-hydroxy-eicosatetraenoic acid (12-HETE) were identified in murine arteries. However, the specific LO responsible for the synthesis of these metabolites was not found in vascular endothelium or smooth muscle. The amount of 12-LO metabolites synthesized depended on the mode of sample preparation. The synthesis of [14C]-AA metabolites was dramatically increased in buffer rinsed, control aortas when compared to heparin-rinsed aortas. We hypothesized that adherent platelets (PLs) are responsible for the synthesis of vasoactive 12-LO metabolites in mouse arteries. Aortic PL adherence and removal of PLs by heparin rinsing were confirmed by immunohistochemical and western immunoblot analysis using specific anti-PL-12-LO and gpIIb/IIIa antibodies. In the presence of indomethacin and L-NA, acetylcholine caused concentration-dependent relaxations in rinsed, control abdominal aortic rings that were preconstricted with thromboxane-mimetic, U46619 (maximum relaxation at 86.4±3.6%) or phenylephrine (maximum relaxation at 81.7±4.1%); however, those relaxations were significantly reduced in heparin-rinsed aortas to 71.6±4.5% (p<0.05) and 48.3±7.2% (p<0.005), respectively. Isolated PLs and rinsed, control aortas showed identical [14C]-AA metabolic patterns. Mass spectrometric identification of AA metabolites produced by rinsed, control aortas or isolated PLs revealed an identical composition consisting of 12-HETE, trioxilin A3, trioxilin C3 and hepoxilin A3. Synthetic trioxilin A3, trioxilin C3 and hepoxilin A3 (3 μM) relaxed U46619-preconstricted mesenteric arteries with maximum relaxations of 78.9±3.2; 82.2±5.0 and 20.0±5.1, respectively. These data indicate that adherent PLs contribute to vascular relaxation by producing PL-12-LO-derived relaxing factors. This effect should be taken into consideration when performing functional studies in isolated mouse arteries. Heparin rinsing of isolated arteries is important to remove adherent PLs and eliminate their contributions to vascular tone.
4/7

THYMOQUINONE CAUSES ENDOTHELium-DEPENDENT AUGMENTATION OF CONTRACTION DEPENDING ON ACTIVATION OF SOLUBLE GUANYLYL CYCLASE IN ISOLATED ARTERIES

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Introduction: Experiments were designed to determine the effects of thymoquinone, an alkaloid with in vivo vasodilator properties, in isolated arteries.

Methods: Rings, with or without endothelium, of rat mesenteric arteries, rat aorta and porcine coronary arteries were suspended in conventional organ chambers for isometric tension recording. Certain rings were incubated with inhibitors of nitric oxide (NO) synthase inhibitor (L-N^G-nitroarginine methyl ester, L-NAME) or soluble guanylyl cyclase (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one, ODQ). They were contracted with phenylephrine (rat arteries) or prostaglandin F_2alpha (porcine coronary arteries) and exposed to increasing concentrations of thymoquinone.

Results: Thymoquinone caused a sustained further increase of tension in rings with endothelium. This augmentation was prevented by endothelium-removal, L-NAME and ODQ. Incubation with the NO-donor detaNONOate in L-NAME-treated rings restored and even increased the contractile response to thymoquinone. By contrast, treatment with 8-bromo cyclic GMP of ODQ-treated preparations did not restore the augmentation by thymoquinone.

Conclusion: These findings demonstrate that thymoquinone causes an endothelium-dependent augmentation similar to that seen in hypoxia (Chan et al., Am J Physiol: H2313, 2011). This facilitation also requires endothelium-derived NO and activation of soluble guanylyl cyclase, but not the presence of cyclic GMP.

4/8

DIFFERENTIAL MECHANISMS OF VASODILATOR EFFECT OF NITRITE IN NORMOTENSIVE AND HYPERTENSIVE RAT AORTAS

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Introduction: A growing body of recent data indicate that dietary inorganic nitrates causes vasodilation leading to lowering of blood pressure in the body; however, the precise mechanisms of nitrite-induced vasodilation remain unclear. Herein, therefore, we studied the mechanisms of nitrite-induced vasodilation in vitro in an isolated tissue bath experiment.

Method: Relaxation responses to sodium nitrite (NaNO_2, 10^-9 to 10^-3 mol/L) were measured in phenylephrine pre-contracted aortic rings isolated from gender- and age-matched normotensive Wistar-Kyoto and spontaneously hypertensive rats in the presence or absence of various pharmacological agents.

Results: Sodium nitrite elicited relaxation in both normotensive and hypertensive tissues, but with a significantly higher potency in the latter tissues. Inhibition of endothelial nitric oxide synthase (NOS) enzyme activity decreased nitrite-induced relaxations in normotensive, but not in hypertensive, tissues. Inhibition of cyclooxygenase (COX) enzyme activity increased relaxation responses to nitrite in both types of tissues. Free radical scavenging with ascorbic acid did not alter responses to nitrite in both types of tissues. Inhibition of NADPH oxidase enzyme activity did not alter responses to nitrite in normotensive tissues but decreased in hypertensive tissues bringing the responses to a level that is seen in untreated control normotensive tissues.

Conclusion: Our findings indicate that 1) nitrite causes varying degree of vasodilation in normotensive and hypertensive tissues with higher response in the latter tissues, 2) COX-derived substances antagonizes relaxations to nitrite, and 3) differential mechanisms (i.e., NOS in normotensive tissues and NADPH in hypertensive tissues) may participate in nitrite-induced relaxations in normal and hypertensive vasculature.
MECHANISMS OF VASCULAR SMOOTH MUSCLE RESPONSE TO ADENOSINE: THE ROLE OF INTACT ENDOTHELIUM

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Introduction: Adenosine is an endogenous purine nucleoside with wide distribution in almost every tissue. It has a minimal concentration in body fluids, yet in conditions associated with tissue ischemia or anoxia, the concentration of adenosine rapidly increases. Adenosine-induced effects are due to the initial activation of A(1), A(2A), A(2B), and A(3) or adenosine receptors. Adenosine has a notable role in regulating different cardiovascular functions. In most animal models an administration of adenosine results in a profound hypotension. Vasodilatations in response to adenosine are predominantly due to direct activation of adenosine A(2) receptors located on vascular smooth muscle cells. However, in many blood vessels adenosine induces indirect action after activation of adenosine receptors on endothelial cells.

Method: The aim of this investigation was to summarize current findings related to mechanisms included in adenosine-induced action on different isolated blood vessels and various vascular beds. The evidence acquisition involved the MEDLINE data base screening and the selection of data with clinical relevance published in the last ten years.

Results: In coronary arteries A(2A) receptors are essential for coronary blood flow regulation. Dilatations of coronary arteries in the presence of adenosine have been described to be both endothelial-dependent and -independent. Myocardial protection during ischemia by adenosine has been associated with activation of ATP-dependent potassium channels on smooth muscle cells.

In the renal circulation the rate of adenosine formation is enhanced when the rate of ATP hydrolysis prevails over the rate of ATP synthesis. Adenosine lowers glomerular filtration rate by constricting afferent arterioles by activation of A(1) receptors. Oppositely, adenosine induces vasodilatation in the deep cortex. This underlines an important role of adenosine in the intrarenal metabolic regulation. Adenosine-induced vascular relaxation is mediated via endothelial A(2A) receptors with subsequent release of endothelial nitric oxide or epoxyeicosatrienoic acid(s).

The blood flow in skeletal muscle is directly associated with metabolic demand. In exercising muscle adenosine acts on extracellular A(2A) receptors located on the vascular smooth muscle and it is associated with preconditioning phenomenon regarding the claudications.

In consideration to cerebral circulation, adenosine is released from the cortex in response to hypoxia/ischemia. A major role of A(2A) receptor occupancy is associated with activation of ATP-sensitive and Ca-activated potassium channels.

Conclusion: So far, endothelium-independent and endothelium-dependent relaxant effects of adenosine have been reported. The signaling pathway, after binding to specific adenosine receptor, would involve release of endothelial relaxing factor(s) and/or opening of specific potassium channels on vascular smooth muscle cell.

AMP-ACTIVATED PROTEIN KINASE (AMPK) DILATES MICROVESSELS VIA ACTIVATION OF THE BKCA CHANNEL

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Introduction: Microvascular perfusion is essential for adequate organ function and is tightly controlled by various mechanisms. Here, we studied whether AMP-activated protein kinase (AMPK) which has been shown to modify vascular tone of large vessels plays a role in microvascular diameter control. The latter may be important especially under pathophysiologic conditions since AMPK activity has been shown to change e.g. under conditions of diabetes or hyperlipidemia.

Method: We conducted smooth muscle calcium and outer diameter measurements in intact segments of freshly isolated skeletal muscle resistance arteries (hamster; n=60). In addition patch clamp studies were performed in smooth muscle cells freshly isolated from these arteries. The isolated vessels were pre-treated with the NOS-inhibitor L-NAME (30 μM), the COX-inhibitor indomethacin (30 μM) and Fura2-AM (2 μM) was used as calcium-indicator.

Results: The AMPK-stimulators A769662 (A76) and PT-1 induced a dose-dependent and endothelium-independent vasodilation which was associated with a decrease in [Ca^{2+}]_i in vessels pre-constricted with norepinephrine (NE, 0.3 μM). In contrast, in vessels pre-constricted by high extracellular potassium (100 mM) or TEA (60 mM) A76 did not induce any [Ca^{2+}]_i decrease. Patch clamp studies revealed activation of BK channels (Maxi-K) by A76 and PT-1, which could be blocked by the specific BK channel inhibitors Paxilline (500 nM) and Iberiotoxin (100 nM) and also by the AMPK-inhibitor Compound C (100 mM). Accordingly, Paxilline and Iberiotoxin (1 μM) inhibited the A76 dilator effect (100 μM) of microvessels preconstricted with NE, though only by about 30%. Likewise, transfection of the vessels with siRNA against the beta1 subunit of the AMPK (the cellular target of A76) also showed a reduction of the A76 induced dilation by about 30%.

Conclusion: AMPK is a potent modulator of microvascular smooth muscle tone. Augmentation of the BK channel current in vascular smooth muscle appears to be an essential mechanism of action of this enzyme, though an inhibition of such channels has been described in other tissues.
ENDOGENOUS UREA – A MODULATOR ENHANCING ALPHA AND ANTAGONISING BETA ADRENERGIC RECEPTORS IN MAMMALIAN CARDIOVASCULAR SYSTEM

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The problem about the differential modulation of alpha- and beta-adrenergic responses is still not elucidated.

Our experiments with uretan-anaesthesized male cats / dogs, under the influence of 17 mmol/kg single urea infusion, shows to 4-time increased initial blood urea level, at the end of 1st, 2nd, 3rd, 4th and 5th hour. In spite of these results there was a dose- and time-dependent diminishing of both the positive chronotropic and vasodepressor effects of isoproterenol (a nonselective beta-adrenergic agonist) 1-2-4 nmol/kg i.v., simultaneously registered. All these results stimulated us for in vitro study of the matter. We used (according to R. FURCHGOTT, 1968; 1972) an optimal concentration 1.6 mmol/l phenolamine in Krebs solution for 60 - 90 min exposure time, without washing out of Krebs solution. We preferred for 60 minutes exposure time. We used urea concentrations 5 or 50 mmol/l in different series, which is correspondent to the physiological and pathological status. All the experiments were performed under isometric regimen cDRC with noradrenaline 30-60-120-240-360 mmol/l. The most important result of our investigation shows that the stability of the complex noradrenaline-alpha-adrenergic receptor in the presence of 5 or 50 mmol/l urea is stable and in part significantly enhanced. The same result is visible with the method of Lineweaver-Burk (1934) for double reciprocal plot graphically. On the contrary, the same urea concentrations, in parallel series using 2 nmol/l butoxamine (a selective beta 2-adrenergic receptor blocking agent), on cDRC with isoproterenol, ranging 0.005-5 nmol/l (precontracted with serotonin rat aortic rings) urea is acts as a significant antagonist. A pure noncompetitive antagonism is demonstrated in Lineweaver-Burk graphs.

Objective: To determine whether or not H2S, a novel endogenous gasotransmitter, attenuates endothelial dysfunction in renovascular hypertensive rats, and explored the underlying mechanisms may be involved.

Methods: Sprague-Dawley rats were randomly divided into three groups: Sham, two kidney one-clip (2K1C), 2K1C+NaHS (sodium hydrosulfide, a H2S donor). Seven-week-old male Sprague-Dawley (SD) rats were anesthetized with intraperitoneal injections of pentobarbital sodium (30mg/kg). In 2K1C+NaHS group (the rats were injected intraperitoneally with NaHS 56 μmol/kg daily from the third day after the 2K1C operation). In the 2K1C and 2K1C+NaHS rats, the left kidney was exposed through a lumbar incision and the right renal artery was clipped. The right kidney was left untouched. The sham procedure was performed the entire surgery as 2K1C rats except that no clip was inserted. The sham rats were kept in cages after surgery, fed with normal chow and given tap water. NaHS was administered daily via intraperitoneal injection at a dosage of 56 μmol/kg starting from the third day after the induction of renovascular hypertension by 2K1C. Sham and 2K1C rat were received vehicle (saline) treatment. To examine the hypertensive effect of H2S, the angiotensin-converting enzyme (ACE) inhibitor captopril (5 mg/kg) was applied daily to 2K1C rats intraperitoneally for 4 weeks. The systolic blood pressure (SBP) was measured before the operation and each week thereafter with a noninvasive tailcuff plethysmograph. Isometric force study was performed on isolated thoracic aorta in organ bath. Western blot was used to determine the protein expression of angiotensin II type1 receptor (AT1R), p67phox, Nox2, Nox4 and superoxide dismutase 1 (SOD-1). The malondialdehyde (MDA) concentration and SOD activity were tested by ELISA. The plasma concentration of angiotensin II (Ang II) and H2S were studied as well.

Results: The SBP and plasma concentration of Ang II were significantly increased, while the plasma level of H2S was decreased in 2K1C rats, which were reversed by NaHS chronic treatment. NaHS supplementation eliminated Ang II-induced vasoconstriction and restored ACh-induced vasorelaxation. AT1R, p67phox, Nox2, and Nox4 protein expression were downregulated by chronic treatment with NaHS. The content of MDA in thoracic aorta was normalized, whereas the activity and protein expression of SOD was elevated.

Conclusions: H2S improves endothelial function in renohypertensive rats. The protective effect of H2S may attribute to the downregulation of AT1R protein expression and decreased oxidative stress.
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