Endothelial dysfunction – a key player in hypertension?

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History of endothelium studies

1772 - Joseph Priestly - NO was discovered

1865 – W. His - used the term “endothelium” to distinguish it from the “epithelium” and to denote the lining of blood vessels and mesothelium-lined body cavities (pleura, pericardium, peritoneum).

1976 – Gryglewski, Bunting, Vane and Moncada, discover prostacyclin

1979 – Gruetter - delivering a gaseous mixture of NO into an organ bath containing isolated pre-contracted strips of bovine coronary artery led to the discovery of the vascular smooth muscle relaxant properties of NO.

1980 - Furchgott and Zawadzki - endothelial cells produce an endothelium-derived relaxing factor (EDRF) in response to stimulation by acetylcholine in vessels with intact endothelium

1986 - Furchgott and Ignarro proposed that the endothelial mediator involved in endothelium-dependent relaxation is nitric oxide (NO).

1987 - Moncada and coworkers demonstrated that indeed NO is a major endothelium-derived relaxing factor.

1988 – Moncada - NO is synthesized from the amino acid L-arginine

1992 - NO as the molecule of the year on the cover of Science magazine

1998 - the importance of the NO discovery was recognized by awarding the Nobel Prize in Physiology and Medicine to Furchgott, Ignarro and Murad.
Endothelium dysfunction insights in physiology and pathophysiology
Structure of arterial wall

Effects of nitric oxide (NO) vs. actions of oxidized LDL

D. Gradinaru et al. / Mechanisms of Ageing and Development 151 (2015) 101–113

Vasodilatation
Growth inhibition
Anti-apoptotic
Anti-thrombotic
Anti-inflammatory
Antioxidant

Vasoconstriction
Growth stimulation
Pro-apoptotic
Pro-thrombotic
Pro-inflammatory
Pro-oxidant

Vascular endothelium
Endothelial dysfunction causes, mechanisms, consequences and therapeutic options

- Hyperglycemia
- Hyperlipidemia
- Hyperinsulinemia
- Insulin resistance
- Hypertension
- Mental stress
- Aging
- Anticancer drugs

ENDOTHELIAL DYSFUNCTION decreased NO synthesis, release and/or activity

- Thrombosis
- Inflammation
- Vasoconstriction

Cardiovascular drugs
- Anti-inflammatory therapy
- Epigenetic therapy
- Exercise
- Diet
- miRNA therapy

Fig. 5. Nitric oxide (NO\(^-\)) produced by cytochrome P450 reductase (CPR) from nitrate. The endothelium-derived NO produced by CPR from nitrate is a supplement for NO produced by eNOS in arteries of the spontaneously hypertensive rat, and reduces the contractions to agonists. However, reactive oxygen species (ROS) significantly eliminate the NO production thus restoring the contractions.
Regulation of vascular tone by nitric oxide (NO)
Effects of lenvatinib (an oral multi-kinase inhibitor) on systolic BP

Lenvatinib (Lenvima®), an oral multi-kinase inhibitor, is effective in the treatment of differentiated thyroid carcinomas (DTCs).

A severe adverse effect of lenvatinib is hypertension, thus limiting its use as an anti-cancer treatment.

All of the 10 patients treated with lenvatinib exhibited significant hypertension.

*D. Sueta et al. / Atherosclerosis 260 (2017) 116–120*
Effects of lenvatinib (an oral multi-kinase inhibitor) on serum nitrogen oxide and plasma vascular endothelial growth factor concentrations

*Sueta et al. / Atherosclerosis 260 (2017) 116–120*
Proposed involvement of oxidative stress, inflammation & antioxidants at vascular level

H.N. Siti et al. / Vascular Pharmacology 71 (2015) 40–56
Effects of different stimuli (physical or chemical) on vascular endothelium

Tousoulis D, Heart 2005, 91,553
The endothelial glycocalyx

Structural diagram of the ESL.

The structure of the glycocalyx

The structure of the glycocalyx

Mechanism of glycocalyx degradation
Proheparanase is released by activated endothelial cells and activated platelets and by secretion from leucocytes. Proheparanase is cleaved into active heparanase by cathepsin L. Heparanase consequently cleaves heparan sulfate in the glycocalyx. Released hyaluron and heparan sulfate fragments promote inflammation. Remodelling of the glycocalyx facilitates endothelium–leucocyte interaction.
Loss of the glycocalyx, therefore, can also increase transcapillary albumin transport and filtration of lipoproteins towards the subendothelial space in tissues. For example, confocal microscopy of the common and internal carotid arteries showed that regions that are prone to atherosclerosis were characterized by reduced endothelial glycocalyx thickness, increased transendothelial transport of LDL, and intimal accumulation of LDL.

**Figure 4** Development of atherosclerotic lesions after loss of the glycocalyx.
ApoE/LDLR^{-/-} mice

ECs elasticity

Glx length

Glx coverage

ApoE/LDLR^{-/-} apolipoprotein E/low-density lipoprotein receptor-deficient mice

ECs elasticity

elasticity of endothelial cells

Glx

Glycocalyx
Degradation of Glycocalyx and Multiple Manifestations of Endothelial Dysfunction Coincide in the Early Phase of Endothelial Dysfunction Before Atherosclerotic Plaque Development in Apolipoprotein E/Low-Density Lipoprotein Receptor-Deficient Mice

ApoE/LDLR −/− apolipoprotein E/low-density lipoprotein receptor-deficient mice

SDC-1 syndecan-1

ESM-1 endocan

sFLT-1 soluble form of fms-like tyrosine kinase

Angpt-2 angiopoietin 2

Anna Bar, *J Am Heart Assoc.* 2019
ApoE/LDLR -/- apolipoprotein E/low-density lipoprotein receptor-deficient mice

sVCAM-1 soluble vascular cell adhesion molecule 1

sICAM-1 soluble intercellular adhesion molecule 1

sE-sel soluble form of E-selectin

vWF von Willebrand factor

t-PA tissue plasminogen activator

PAI-1 plasminogen activator inhibitor 1
Using a novel method combining Raman spectroscopy for biochemical analysis and Atomic Force Microscopy (AFM) for analyzing the endothelial nanomechanics an increased intracellular lipid content and elevated cortical stiffness/elasticity were shown in ApoE/LDLR−/− aortas.
To activate single glycocalyx components within a robust layer, atomic force microscopy (AFM) was used in combination with specific antibodies to “pull” on individual proteins or GAGs. AFM pulling on glypican-1 and heparan sulfate for 10 min caused significantly increased NO production.
Schematic of factors that can contribute to hypertension-associated vascular changes

- Ang II, ET-1
- RAAS Activation
- Salt
- Lifestyle factors

- Apoptosis
- Proliferation
- Arterial stiffness
- Fibrosis
- Vascular inflammation
- Oxidative stress
- NO bioavailability
- Vasodilatation
- Vasoconstriction

- Remodelling
- Calcification
- Endothelial dysfunction

- Hypertension
Endothelium dysfunction as cardiovascular risk factor
Participants 2 051 158 participants (54% women) from general population cohorts (n=1 861 052), high risk cohorts (n=151 494), and chronic kidney disease cohorts (n=38 612).

Results Risks of all-cause mortality and cardiovascular mortality were higher in men at all levels of estimated glomerular filtration rate and albumin-creatinine ratio. Compared with a urinary albumin-creatinine ratio of 5, the adjusted hazard ratio for all-cause mortality at urinary albumin-creatinine ratio 30 was 1.69 in women and 1.43 in men.

Conclusions Both sexes face increased risk of all-cause mortality, cardiovascular mortality, and end stage renal disease with lower estimated glomerular filtration rates and higher albuminuria.
Association between albuminuria and cerebral small vessels disease (A) white matter hyperintensities, (B) lacunar infarcts

### A

<table>
<thead>
<tr>
<th>Study (Author, year)</th>
<th>N population</th>
<th>N events</th>
<th>OR (95% CI)</th>
<th>% Weight</th>
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<tbody>
<tr>
<td>Hayashi, 2017</td>
<td>1716</td>
<td>239</td>
<td>0.71 (0.24, 1.70)</td>
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<td>Suda, 2017</td>
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<td>Tamura, 2016</td>
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<td>Vilar-Bergua, 2016</td>
<td>975</td>
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<td>Akoudad, 2015</td>
<td>2526</td>
<td>631</td>
<td>1.29 (0.83, 1.98)</td>
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<td>Cho, 2012</td>
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<td>de Bresser, 2010</td>
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<td>Henskens, 2009</td>
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<td>Tanaka, 2009</td>
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<td>Weiner, 2009</td>
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<td>Anan, 2008</td>
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<td>Barzilay, 2008</td>
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<td>Knopman, 2008</td>
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<td>Wada, 2007</td>
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<td>Overall (I-squared = 43.7%, p = 0.025)</td>
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<td>1.70 (1.43, 2.01)</td>
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<td>Hashimoto, 2008</td>
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<td>1.89 (1.08, 3.31)</td>
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<td>Wada, 2007</td>
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<td>1.53 (1.02, 2.30)</td>
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<td>Yoshikawa, 2007</td>
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<td>2.24 (0.96, 5.21)</td>
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<td>Ravera, 2001</td>
<td>22</td>
<td>12</td>
<td>12.00 (1.58, 91.09)</td>
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<td>Overall (I-squared = 26.7%, p = 0.182)</td>
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<td>1.86 (1.49, 2.31)</td>
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Flow-mediated dilation among 8 groups defined by systolic BP and use of antihypertensive drug

FMD-J Study (Flow-Mediated Dilation Japan) Hypertension. 2017;70:790-797
Brachial flow-mediated dilation predicts target organ damage progression
Brachial flow-mediated dilation predicts target organ damage progression

Yang et al.  Journal of Hypertension 2014, 32:2393–2400
The meta-analysis found that both brachial FMD and digital RH-PAT have significant predictive value for future cardiovascular events after adjustment for other risk factors.

The prognostic magnitudes of these 2 methods in CVD population were similar, and a 1 SD increase or decrease was associated with 50% lower risk or doubled risk of cardiovascular events.

Future studies should explore whether endothelial function-guided therapies provide benefits in improving cardiovascular outcomes.
Thrombomodulin as a New Marker of Endothelial Dysfunction in Chronic Kidney Disease in Children

![Graph 1: BNP (pg/ml) vs. Thrombomodulin (ng/ml)](R = 0.406; p = 0.001)

![Graph 2: SBP-24h (mmHg) vs. Thrombomodulin (ng/ml)](R = 0.345; p < 0.011)

![Graph 3: cLDL (U/L) vs. Thrombomodulin (ng/ml)](R = 0.34; p < 0.009)

![Graph 4: FVIII (mg/mL) vs. Thrombomodulin (ng/ml)](R = 0.293; p < 0.024)
Endothelium dysfunction prophylaxis and treatment
Therapeutic approaches to promote vascular health and improve vascular function in hypertension
## Antihypertensive drugs and beneficial vascular effects

<table>
<thead>
<tr>
<th>Drug class</th>
<th>Example drugs</th>
<th>Possible beneficial vascular effects</th>
</tr>
</thead>
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<tr>
<td>ACE inhibitors</td>
<td>Lisinopril, Perindopril, Enalapril, Ramipril</td>
<td>↑ NO bioavailability, ↓ Production of reactive oxygen species, Vasodilation, anti-inflammatory</td>
</tr>
<tr>
<td>Angiotensin II receptor blockers</td>
<td>Losartan, Valsartan, Candesartan</td>
<td>↑ NO bioavailability, ↓ Production of reactive oxygen species, Vasodilation, anti-inflammatory</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>Amlodipine, Lercanidipine, Nifedipine</td>
<td>Improved cellular redox state</td>
</tr>
<tr>
<td>Mineralocorticoid receptor blockers</td>
<td>Spironolactone, Eplerenone</td>
<td>↓ Pro-inflammatory/pro-fibrotic changes</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>Nebivolol, Carvedilol</td>
<td>↑ NO bioavailability, Reactive oxygen species scavenger</td>
</tr>
</tbody>
</table>

*ACE* angiotensin converting enzyme, *NO* nitric oxide, ↓ decrease, ↑ increase

A. C. Cameron et al. Drugs (2016) 76:1529–1550
**FIGURE 1** BP-Independent Effects of High Dietary Sodium

**Brain**
- sensitized sympathetic neurons

**Heart**
- increased left ventricular hypertrophy

**Kidney**
- decreased glomerular filtration rate
- increased protein excretion

**Blood vessels**
- impaired endothelial function
- increased arterial stiffness

**BP INDEPENDENT EFFECTS OF HIGH DIETARY SODIUM**

**Suboptimal Consumption of Fruits & Vegetables & Consequent Suboptimal Consumption of Dietary Potassium & Antioxidants**

- Increased Superoxide & Other Reactive Oxygen Species & Nitrogen Species & Increased Intracellular Acidity

- Oxidative Stress & Acidotic Stress & Diminished Nitric Oxide Bioavailability

- Vascular Endothelial Dysfunction

- Failure of Vascular Smooth Muscle Relaxation

- Hypertension

**Supraphysiological Consumption of Dietary Sodium**
High salt impairs endothelial function.

Under low salt conditions, the soft endothelial cortex is easily deformable, the amount of shear stress-induced NO release is high, and the vascular smooth muscle cells relaxed.

In the case of high salt-induced cortical stiffening, the NO release is reduced, leading to contracted smooth muscle cells and vasoconstriction.

EnNaC endothelial Na+ channel
eGC endothelial glycocalyx
Dietary Intake of Fruits and Vegetables Improves Microvascular Function in Hypertensive Subjects in a Dose-Dependent Manner

Figure 1. Overview of study design.

Figure 3. Change in maximum response to acetylcholine plotted against change in daily fruit and vegetable intake.

Damian O. McCall  Circulation. 2009;119:2153-2160
Several polyphenol-rich natural products and polyphenols have been shown to improve vascular ageing by reducing endothelial dysfunction, platelet activation, vascular oxidative stress, over-activation of the local angiotensin system and increased pro-thrombotic responses associated with cardiovascular risk factors in preclinical and clinical studies.
Alpha-lipoic acid (ALA) is a dietary supplement exerting anti-oxidant and anti-inflammatory effects.

At three months, within the ALA and placebo groups, FMD did not change significantly. However, the basal and peak diameter of brachial artery significantly increased after ALA treatment as compared to placebo ($p = 0.036$ and $p = 0.01$, respectively)

**Figure 2.** Change in basal (A) and maximal (B) brachial artery diameter at three-month follow-up.
Fig. 4. The majority of exercise effects on the vascular endothelium are mediated by intermittent increases of laminar shear stress. On the luminal side of the endothelial cells, direct signaling can occur through deformation of VEGF receptor 2 (VEGFR2) or platelet endothelial cell adhesion molecule 1 (PECAM1) activating phosphatidylinositol 3-kinase (PI3K) to phosphorylate Akt (PKB) and induce Akt-mediated endothelial nitric oxide (NO) synthase (eNOS) phosphorylation, leading to higher NO production. PDK, phosphoinositide-dependent kinase.
Endothelial dysfunction

- is associated with almost all cardiovascular risk factors
- precedes the development of atherosclerosis
- predicts cardiovascular events independently of classical risk scores
- might identify a subset of patients in which conventional treatment is not sufficient
- accompanies prehypertension
Endothelial dysfunction

While many systems contribute to blood pressure elevation, the vascular system is particularly important because vascular dysfunction is a cause and consequence of hypertension.

Pharmacological substances, which are able to improve endothelial nanomechanics and function, could take a new importance in the prevention and treatment of vascular diseases.

Antihypertensive drugs that influence vascular changes associated with high BP have greater efficacy for reducing cardiovascular risk than drugs that reduce BP, but have little or no effect on the adverse vascular phenotype.

Increased physical activity and consumption of fruit, vegetable and fish improves endothelial function and reduces BP and should therefore be strongly encouraged in both the general population and patients with hypertension.