Chapter

# Endothelial Dysfunction, Molecular Biology, Physiopathology, Diagnosis, and Treatment

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## Abstract

Endothelial cell dysfunction has lately become one of the principal subjects being incorporated into the assessment of cardiovascular risk because of the relevance that has been shown in several clinical studies. Comprehending and incorporating basic physiological knowledge, about endothelium molecular biology and vascular tonicity, is key to understanding the relevance of this topic. The approach of endothelial dysfunction physiopathology is overly complex and widely studied, but it can be enrolled into both consumption of bioavailable NO and deficit production of NO. In the last decades, scientific equipment has been developed from the necessity of creating non-invasive tools to measure arterial stiffness, being FMD one of the first and most used ones. Once the endothelial cell dysfunction was identified, several drugs and bioactive substances were evaluated because of their potential to decrease the level of arterial stiffness and improve life quality, such as polyphenols, phosphodiesterase five inhibitors, and new incoming therapies.

**Keywords:** endothelial cell dysfunction, nitric oxide, flow mediated dilatation, polyphenols, PDE5i

## 1. Introduction

Endothelial cell dysfunction (ECD) is defined as an altered metabolism of available nitric oxide (NO), or an imbalance of relaxing and constrictor endothelial factors [1]. Many of the physiological functions of the endothelial cells (ECs) are involved with the regulation of vascular tonicity, balancing of blood fluidity and thrombosis through coagulation and fibrinolysis factors, vascular inflammatory and immunological process control, and several growth factors [2]. Any alteration in these systems can lead to a loss of vascular homeostasis and contribute to developing endothelial dysfunction [1, 2].

## 2. Endothelium molecular biology, vascular tonicity and its regulation

Vascular tonicity is regulated by multiple molecules, proteins, hormones, and peptides secreted or with action mechanisms on the ECs such as an atrial natriuretic peptide, eicosanoids, adrenal steroids, sodium, and water excretion, and reno-medullary endothelial systems [3].

Examples of several endothelium-derived hyperpolarizing factors are NO and prostacyclin, whereas endothelin-1 (ET-1), angiotensin II, thromboxane A2 and reactive oxygen species (ROS), relaxing factors [4].

### 2.1 Nitric oxide (NO)

NO is a reactive, diffusible gaseous free radical whit strong intrinsic oxidant properties. It is produced locally at ECs by three different isoforms of NO synthase (NOS) enzymes, each with unique expression and functional properties: neuronal NOS (nNOS, NOS1), inducible NOS (iNOS, NOS2), and endothelial NOS (eNOS, NOS3) [5].

Elevated levels of intracellular Ca2+, acting through calmodulin, activates nNOS and eNOS respectively; iNOS is less susceptible to Ca2+, but around 1000 times more inducible by inflammatory stimuli such as TNF- α, IL-1β, and IFN-γ [6].
The NOS produced NO by catalyzing the oxidation of the nitrogen guanide of the L-arginine and $O^2$ producing L-citrulline and NO (**Figure 1**) [5, 6]. The NO activates soluble guanylyl cyclase (sGC), which at binding creates an augmentation of the Vmax of sGC and, consequently, rising the cellular cyclic guanosine monophosphate (cGMP) [6].

The cGMP vascular effects are mediated by several mechanisms, being the activation of protein kinase G (PKG) one of the main processes, conducting vasodilatation by means of release inhibition of Ca2+ mediated by inositol 1,4,5-trisphosphate (IP3) [6].

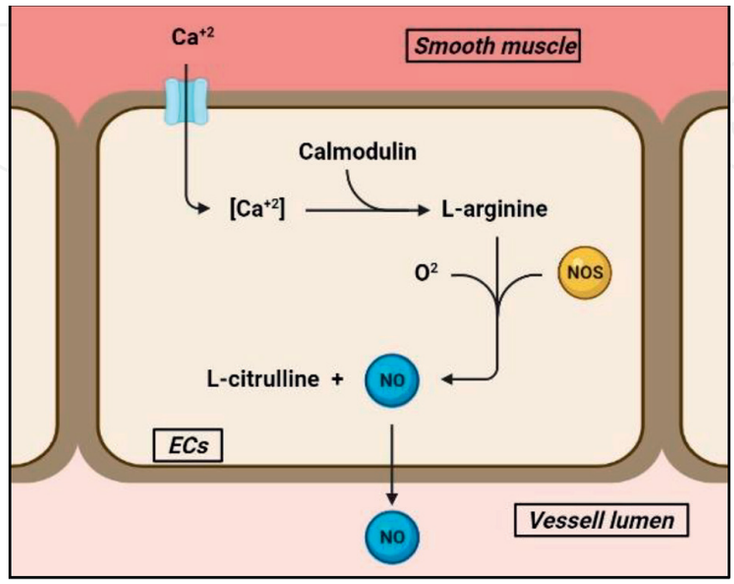


**Figure 1.**
*The graphic shows the activation of NOS mediated by Calmodulin/$Ca^{+2}$. Subsequently, NOS produced NO and L-citrulline starting from L-arginine and $O^2$. Original graphic created with BioRender.com.*

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### 2.2 Endothelin-1 (ET-1)

ET-1 is a peptide of 21 amino acids that has two disulfide junctions, synthesized from a 39 amino acid precursor sequence named pre-pro endothelin by the activity of endothelin-converting enzyme (ECE) (**Figure 2**). ECE-1 restricts the synthesis of ET-1. The ET-1 its produced mainly in ECs, induced by several cytokines, angiotensin II and mechanical stress. It is codified by EDN1 gene, which expression is reduced by NO and prostaglandin I2 [7].

There are two basic types of ET-1 receptors: ETA and ETB. Both receptors are coupled to a G-protein and to the formation of IP3. ETA is, in normal conditions, the most prevalent of these ET-1 receptors [8].

ET-1 action is characterized by vasoconstriction; this effect is initiated once it binds to ETA receptor. The union of these results in the activation of Gq-PLC-IP3 pathway. IP3 induces the release of Ca2+ of the endoplasmic reticule by opening the L-type Ca2+ channels and increasing the cytosolic Ca2+, which produced the contraction of the muscular smooth cells and subsequent vasoconstriction (**Figure 3**) [1, 7, 8].

Despite the presence of ETB receptor on vascular smooth cells, it is also found on ECs, which stimulates the formation of NO causing vasodilatation, and additionally decreases the ET-1 synthesis causing relaxation [1].

## 3. Endothelial dysfunction physiopathology

ECD is defined biochemically by a decreased amount of available NO in the vasculature. There are multiple mechanisms that reduce this value, moreover, the whole dysfunction can be enrolled into two main categories [9].

### 3.1 Consumption of bioavailable NO

Altered NO metabolism due to elevated degradation of NO, inactivation of NO, or presence of NO inhibitors may be due to the elevation in oxidative stress [10]. NO is a highly diffusible and reactive species with an unpaired electron, because of this, there are a variety of chemical components that impede appropriate signaling [11]. Some of the principal agents of this deficiency are ROS and superoxide ($O^{2-}$).

ROS increases the activity of stimulants such as inflammation, radiation, advanced age, obesity, and sundry chemical substances. Superoxide is an important

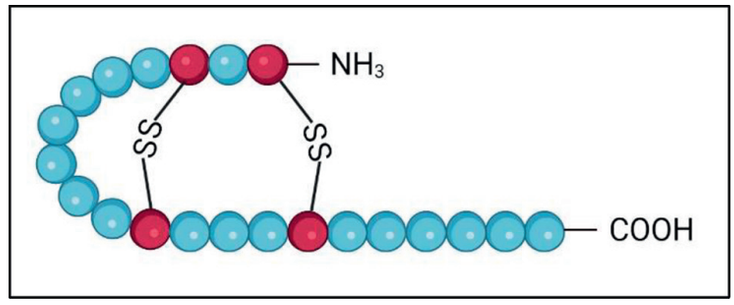


**Figure 2.**
*Amino acid sequence of ET1, characterized by the presence of 21 amino acids and two disulfide junctions. Original graphic created with BioRender.com.*

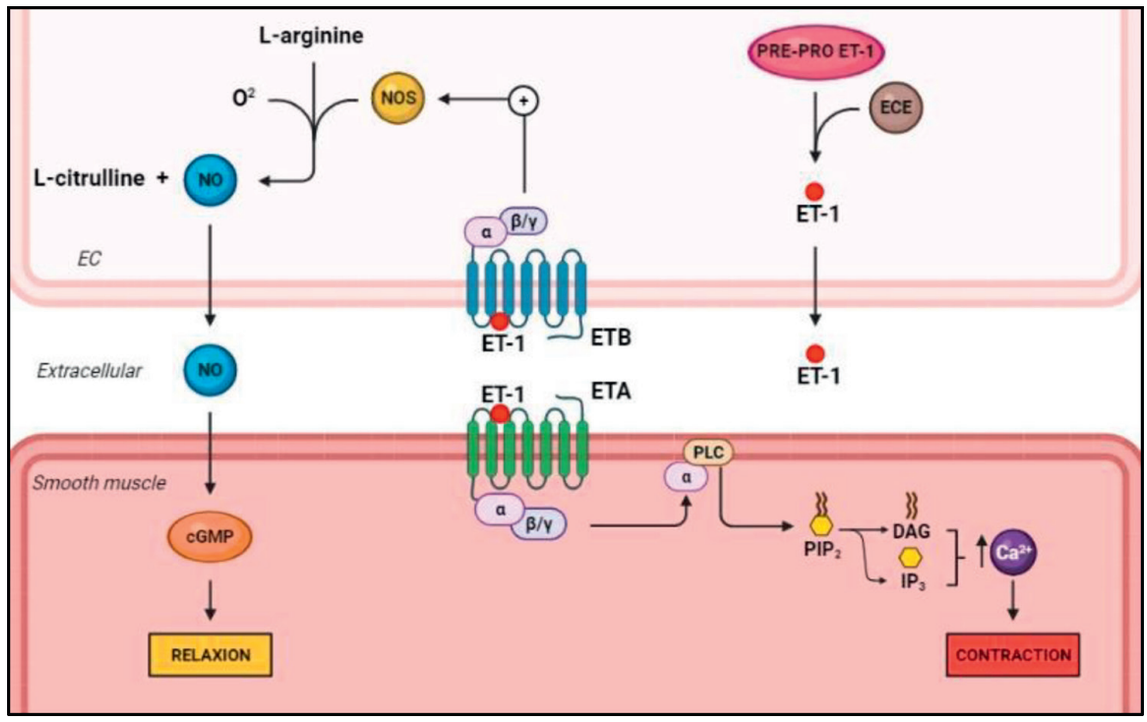


**Figure 3.**
*The ET-1, synthesized from pre-pro ET1 by the activity of ECE in ECs, binds to ETA receptor in vascular smooth muscle and activates the pathway Gq-PLC-IP3, which rises cytosolic Ca2+ and induces muscular contraction. ET-1 can also activate ETB receptor in ECs leading to an increase in NOS activity and augmentation of bioavailable NO. Original graphic created with BioRender.com.*

radical for cardiovascular biology, formed by one-electron reduction of oxygen. At a cellular level, increase oxidative stress causes damage by altering several molecules' structures like deoxyribonucleic acid, proteins, lipids, and carbohydrates [12].

### 3.2 Deficit production of NO

Approaching ECD through the deficit of NO production, modifications of eNOS is one of the processes that stand out in this category, being eNOS uncoupling is a major mechanism. This enzyme requires dimerization in the presence of heme and BH4 for an effective electron movement to L-arginine and the subsequent formation of NO and L-citrulline [3, 4, 13]. When this relation is disrupted, the outcome is that eNOS function as a weak NADPH oxidase, generating $O^{2-}$ instead of NO, a process denominated eNOS uncoupling. Several mechanisms induce eNOS uncoupling, which increases local oxidative stress and removes the vasodilatation effect of NO [13].

Many pathways contribute to eNOS uncoupling, being ONOO one of the main. Also known as peroxynitrite, ONOO is an oxidant and nitrating agent with an unstable structural isomer of nitrate. The formation of this molecule is due to the reaction of free radical superoxide, with free radical nitric oxide. ONOO disrupts a zinc-thiolate cluster in eNOS and oxidizes BH4 to BH3, both creating an eNOS uncoupling and creating a cycle of ROS production [14].

Other, but also well-known, mechanism is L-arginine decrease associated with its inhibitor asymmetric dimethyl-L-arginine (ADMA). ADMA is an endogenous protein produced by N-methyltransferase type 1, elevated in redox status, and degraded by dimethylarginine dimethylaminohydrolase, altered by oxidative stress [15, 16].

## 4. Diagnosis

Cardiovascular disease (CVD) remains as the principal cause of morbidity and mortality worldwide [17]. During last century multiples, studies have been developed with the intention to identify the association between several lifestyle factors and the probability of suffering CVD [18]. Moreover, it is established the presence of cardiovascular risk factors (CVRF) in early childhood is a predictor of CVD in their lifetime [18, 19].

At the end of the last century, some equipments were created and able to identify the endothelium condition through non-invasive tools. Flow-mediated dilation (FMD) has become the most popular and widely used method for examining noninvasive peripheral artery endothelium-dependent dilation [20].

### 4.1 FMD

Flow-mediated dilation represents an endothelium-dependent, largely NO-mediated dilatation of conduit arteries in response to an imposed increase in blood flow and shear stress first described in 1992 [20].

#### *4.1.1 FMD procedure*

FMD is typically assessed in brachial artery with a standardized diameter of 3–5 mm. Through a high-resolution B-mode ultrasound, images of the brachial artery are taken, usually with an ultrasound probe of 7.5–12 MHz [21]. An approach by tangential scanning is a common mistake and results in underestimation of the true brachial artery diameter (**Figure 4**). Recent studies, which adopt H-shaped, probe capturing two short-axis and one long-axis for automatic probe position correction may overcome this previous limitation [22].

A simultaneous evaluation of pulse-wave Doppler velocity is recommended, given the importance of shear stress as the eliciting stimulus for dilatation. The recommended isonation angle is <60° for optimal data acquisition, which should be kept constant [23, 24].

To ensure an optimal image throughout the hole FMD procedure, a probe-holding device is recommended. A stereotactic adjustable probe-holding device allows adjustment of probe position during the test, allowing to maintain the same scan in the study [25].

Many subject-related factors can influence FMD such as alcohol, smoking, food, supplements, drugs, physical activity, and mental stress. Some factors directly stimulated NO-release, but others, such as acute physical exercise and mental stress, modify baseline vasomotor tone [26, 27].

#### *4.1.2 Clinical evidence*

In a study, brachial FMD has associated whit intima-media thickness progression in a population free of CVD, and in hypertensive, postmenopausal women [28]. A follow-up study in hypertensive patients with FMD predicted target organ damage progression for 3 years, even adjusted for known CVRF [29].

One meta-analysis described a significant 8–13% lower risk of CVD per percentage point increase in brachial artery FMD (e.g. from 7–8% dilatation). This reduction

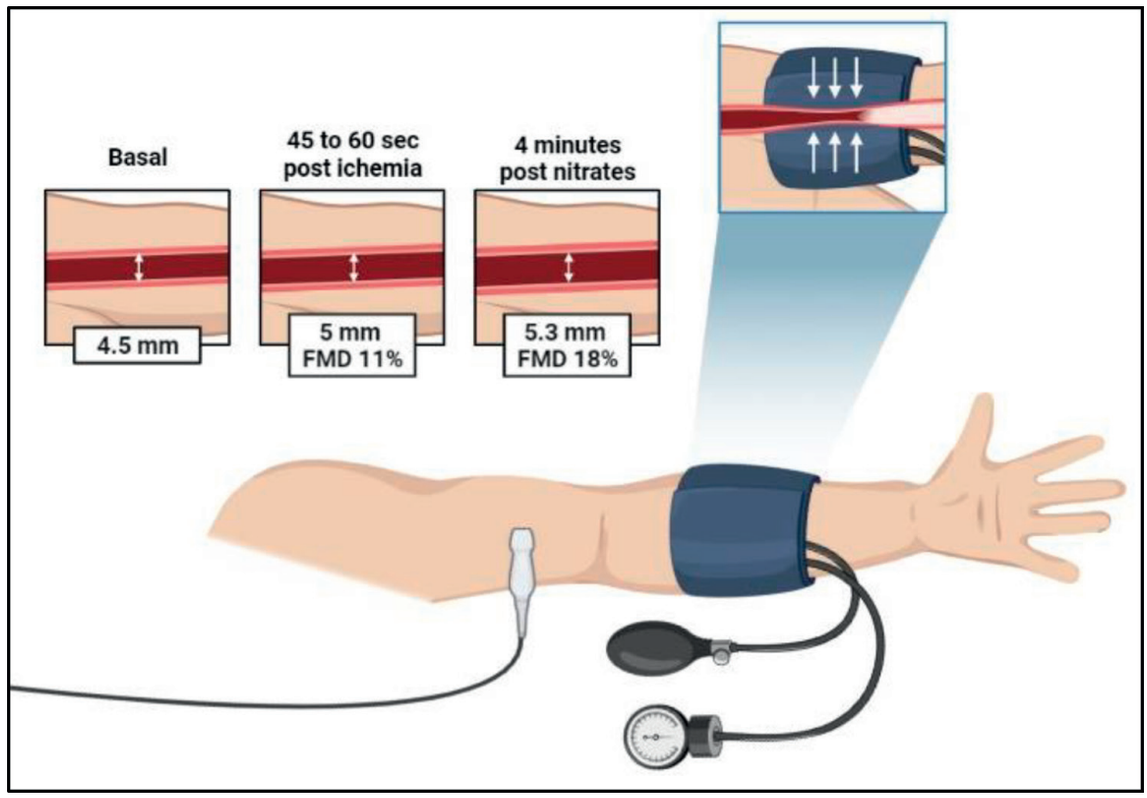


**Figure 4.**
*FMD representation with cuff positioned in the forearm. Through ultrasound assessment, brachial artery diameter is measured before and 5 minutes after the ischemia. FMD can oscillate depending on if a hypertensive drug is used before the procedure such as nitrates. Original graphic created with BioRender.com.*

was present in high and low-risk population but appeared larger in patients with established CVD [30, 31].

The clinical value of long-term changes in FMD may have a prognostic implication. For interventional trials FMD could represent a surrogate endpoint, especially since FMD is a tool with a rapid response effect to therapies, allowing recognition and identification of new bioactive substances or drugs able to modify FMD [32].

## 5. Treatment

### 5.1 Lifestyle

It is well established that lifestyle interventions have a main role in prevention of CVD. Many activities such as diet, aerobic exercise, quitting smoking and alcohol, and a non-sedentary day routine, have shown a significant reduction in blood pressure (BP) and arterial stiffness [33].

#### *5.1.1 Mediterranean diet*

The diet is one of the tractable modifiers of vascular health and BP, which has exhibited that targeting the whole diet has a more significant effect on BP than focusing on individual foods and nutrients [34].

The prevention with Mediterranean-style diet in several trials in patients with high CVRF showed that a Mediterranean diet supplemented with olive oil or nuts, reduced diastolic blood pressure by −1.5 mm Hg and − 0.7 mm Hg respectively, in comparison with low-fat diet over 4 years [35].

The recommendation to incorporate Mediterranean diet for older adults aiming its effect on BP and arterial stiffness is established in a 12-month randomized controlled trial called NU-AGE study. A total of 1294 healthy participants were included, aged 65 to 79 years, recruited from 5 European centers, and arterial stiffness was assessed in 225 participants using the Vicorder device measuring both carotid-femoral pulse wave velocity (PWV) and augmentation index (AIx) [36, 37]. The intervention group received individually tailored standardized dietary advice and commercially available foods to increase adherence to a Mediterranean diet, and the control group continued their habitual diet, and were provided with current national dietary guidance. Of the original sample, 1142 participants completed the trial, and after 1 year, the intervention group resulted in a significant reduction in systolic blood pressure (−5.5 mm Hg; 95% CI, −10.7 to −0.4; P = 0.03), and in a subset (n = 225), augmentation index was improved following intervention (−12.4; 95% CI, −24.4 to −0.5; P = 0.04), with no change in pulse wave velocity [37].

The favorable effects of the Mediterranean diet on health may result from high intake of omega-6 and omega-3 fatty acids, fibers, antioxidants, and polyphenols [38].

#### *5.1.2 Polyphenols*

There are scientific studies that showed polyphenol-enriched diet impedes hyperlipidemia and coronary endothelial dysfunction, both by counteracting vascular inflammation and oxidative damage by activating Akt/eNOS pathway [39]. Some of the polyphenol's effects are linked to the promotion of SIRT1-induced repression of the p38 MAPK/NF-kappaB pathway and ROS production [40].

When they come from virgin olive they reduce inflammatory angiogenesis in ECs through inhibition of matrix metalloproteinase-9 and cyclooxygenase-2, supporting the protective role of dietary polyphenols both in atherosclerosis and cancer [41].

### 5.2 Pharmacological therapy

Several drugs have actions mechanism involved in the physiological pathways of endothelial regulation and vascular tonicity, therefore this section will be discussed briefly a few of them.

#### *5.2.1 PDE5i*

Phosphodiesterase of cyclic nucleotide is a family of enzymes that hydrolyzed the cyclic nucleotides 3′-5′ to their 5′ monophophates analogs [42].

Vardenafil is one of many PDE5i in which a reduction of arterial stiffness has been reported. In one study twelve patients with erectile dysfunction, mean age of 58 ± 9 years, received verdanafil20 mg per day, in a randomized, placebo-controlled, double-blind2-way crossover design. Aortic stiffness was evaluated through carotid-femoral PWV and AIx. PWV decreased significantly (0.7 m/s, P = .001), denoting a decrease in aortic stiffness, and AIx decreased significantly (by 7%, P = .008), denoting a decreased effect of wave reflections from the periphery [43].

### *5.2.2 New therapies*

Recent studies showed that microRNAs have a key role during atherosclerotic plaque formation, representing a potential new target for developing drugs.

In atherosclerotic plaque, miR-143 was found to be upregulated, and its overexpression in human umbilical vein endothelial cells (HUVECs) suppressed glycolysis by targeting hexokinase 2, leading to endothelial dysfunction [44]. And, in vivo, the inhibition of miR-92a, a regulator of endothelial proliferation and angiogenesis after ischemia, results in beneficial effects on the endothelium such as reducing inflammation and decreasing plaque size [45, 46].

Recent evidence shows several epigenetic pathways involved in endothelial dysfunction and related to cardiovascular diseases which will be discussed in the following.

Histone deacetylase 1 (HDAC1) overexpression in bovine aortic endothelial cells triggers a reduction of eNOS lysine acetylation and NO production. Its inhibition can stand as a therapy for preventing endothelial dysfunction. Additionally, HDAC1 decline leads to no change in eNOS acetylation, otherwise increasing basal nitrate NO formation [46, 47].

Another study evidence that resveratrol, a phenol produced naturally by different plants, prevents TNF-α-induced injury from damaging HUVECs by stimulating sirtuin-1 (SIRT1) and repressing p38 MAPK/NF-kappaB pathway and ROS production [40, 46].

Additionally, another NAD-dependent deacetylase, SIRT6 is expressed in atherosclerotic disease in human patients. In several mice studies, absence and haploinsufficient SIRT6 have been associated with monocyte adhesion to endothelium, augmentation of atherosclerosis gene expression, impaired vasorelaxation, and overexpression of VCAM-1 [48, 49]. Being so this knowledge is a potential subject for investigation of novel therapies counteracting atherosclerosis and decreasing endothelial dysfunction.

## 6. Conclusions

ECD is a vast, interesting, and shallow subject shortly explored by the scientific community, therefore, the actual information about this topic is very limited. Further clinical and molecular research should be addressed for a better understanding of the entire implications of these pathways in clinical and molecular investigations.

Moreover, current equipment for addressing clinical non-invasive parameters of arterial stiffness is emerging and earning a relevant place in cardiovascular risk assessment, therefore, these tools are already being incorporated in several international medical guidelines as an important parameter to consider.

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## Conflict of interest

There was no conflict of interest in the making of this document.

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## Appendices and nomenclature

| | |
|---|---|
| ECD | Endothelial cell dysfunction |
| NO | Nitric oxide |
| ECs | Endothelial cells |
| ET-1 | Endothelin-1 |
| ROS | Reactive oxygen species |
| NOS | NO synthase |
| nNOS, NOS1 | Neuronal NOS |
| iNOS, NOS2 | Inducible NOS |
| eNOS, NOS3 | Endothelial NOS |
| sGC | Soluble guanylyl cyclase |
| PKG | Protein kinase G |
| IP3 | 1,4,5-trisphosphate |
| ECE | Endothelin-converting enzyme |
| $O^{2-}$ | Superoxide |
| CVD | Cardiovascular disease |
| CVRF | Cardiovascular risk factors |
| FMD | Flow mediated dilation |
| BP | Blood pressure |
| PWV | Pulse wave velocity |
| AIx | Augmentation index |
| PDE5i | Phosphodiesterase 5′ inhibitors |
| ADMA | Asymmetric dimethyl-L-arginine |
| HUVECs | Human umbilical vein endothelial cells |
| HDAC1 | Histone deacetylase 1 |
| SIRT1 | Sirtuin-1 |



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