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Review Article

The role of nitric oxide in mediating endothelium function in diabetes

Seetha Munisamy[1]*, Hemaniswarri Dewi Dewadas[2], Uma Eswari Punchanathan[3], Vaughn Alexei Ng Bansing[1], Lucas Cashev[1], Kavinash Selva Ganesan[1]

[1]Department of Pharmacology, Faculty of Medicine, Manipal University College Malaysia (MUCM), Jalan Padang Jambu, Bukit Baru 75150, Malacca, [2]Centre for Biomedical and Nutrition Research (CBNR), Faculty of Science, Universiti Tunku Abdul Rahman, Kampar 31900, Perak, [3]Centre for Learning and Teaching (CLT), Universiti Tunku Abdul Rahman, Kampar 31900, Perak, Malaysia

For correspondence: **Email: seetha2009@yahoo.com; **Tel:** + 06-289 6662/3910*

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Abstract

Diabetes mellitus (DM) is one of the leading causes of death. It is associated with cardiovascular diseases and impaired endothelial function, which is critical for cardiovascular health. Endothelial cells in blood vessels release endothelium-derived factors (EDFs) which include endothelium-derived relaxing factors (EDRFs) like prostacyclin, nitric oxide (NO), and endothelium-derived hyperpolarizing factor (EDHF), which relax blood vessels, and endothelium contracting factors (EDCFs) like angiotensin II, endothelin I and thromboxane A2, which cause contractions. Diabetes impairs endothelium-dependent relaxation, largely mediated by NO. Several studies have demonstrated that NO acts as a vasodilator which is essential in mediating endothelium-dependent relaxation. Diabetes treatments today are largely centered on reducing blood sugar levels. However, it is important to understand that endothelial dysfunction (ED) begins as soon as diabetes is diagnosed. This dysfunction is an early warning sign of atherosclerosis, which progresses to cardiovascular disease. This review provides insights into NO and endothelial dysfunction mechanisms, aiding the development of current and future treatments.

Keywords: *Atherosclerosis, Cardiovascular disease (CVD), Diabetes mellitus (DM), Endothelial dysfunction (ED), Nitric oxide (NO)*

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INTRODUCTION

Diabetes mellitus (DM) is rapidly emerging as a significant global health concern, contributing to a substantial number of deaths. The worldwide prevalence of DM has surged, nearly doubling from 4.7 % reported in 1980 to 8.5 % cases in 2014 and is projected to reach 12.2 % by 2045 [1]. Also, DM leads to complications in both micro and macro vessels. Microvascular damage may result in diabetic retinopathy, neuropathy and nephropathy, while macrovascular damage leads to cardiovascular diseases (CVD), peripheral vascular disease and stroke. Risk of death from CVD causes is 2 – 6 times greater for those with diabetes [2]. Cardiovascular disease is typically caused by a condition called atherosclerosis, which involves narrowing and hardening of the blood vessels. Endothelial cells within blood vessels are essential for controlling blood flow to

ensure vascular health. These cells release vasoactive substances, containing endothelium-derived relaxing factors (EDRFs) and endothelium-derived contracting factors (EDCFs), which control constriction and dilation of vessels. Nitric oxide (NO), prostacyclin (PGI2) and endothelium-derived hyperpolarizing factor (EDHF) are examples of EDRFs and they promote vessel relaxation, while EDCFs are responsible for causing vessel contraction. Endothelial dysfunction (ED), recognized as an early marker of atherosclerosis, occurs when there is an imbalance in vasoactive substances due to damage to the endothelium. This leads to decreased EDRF secretion and increased EDCF production. Diabetic patients have been found to have impaired vasodilation in micro and microcirculation [3]. Studies have shown that a decrease in NO bioavailability leads to decreased impairment in NO-mediated relaxation [4]. Nitric oxide is known to mediate relaxation in large arteries, while EDHF predominantly affects small arteries [5]. This review examines the role of nitric oxide (NO) as the primary source of vasodilation, offering insights into the mechanism of erectile dysfunction (ED) and development of current and future treatments.

Endothelium-derived relaxing factors (EDRF)

Nitric oxide (NO)

Nitric oxide has antiatherogenic properties as well as control over endothelium-dependent vasodilation. Reduced vasodilation in large conduit vessels is often associated with defects in NO production or activity, typically resulting from damage to endothelial cells. In intact blood vessels, NO counteracts the effects of EDCF, but endothelial cell injury leads to vasoconstriction. Diminished NO levels contribute to development of atherosclerosis and progression toward CVD (Figure 1).

Endothelial cells continuously produce NO due to their short half-life which is generated from oxidation of L-arginine, along with a by-product called L-citrulline. Its synthesis requires several cofactors, including NADPH, flavin mononucleotide, flavin adenine dinucleotide and tetrahydrobiopterin (BH_4) [6].

Mechanism action of NO

Endothelial nitric oxide synthase (eNOS) mediates blood vessel relaxation in intact blood vessels. Chemical (acetylcholine, bradykinin, etc.) and physical stimuli (shear stress such as reactive hyperemia, laminal stress and pulsatile shear stress) increase calcium levels, triggering eNOS activity and NO release [7]. When NO is produced, it circulates to nearby vascular smooth muscle cells (VSMC) and triggers guanylyl cyclase (GC) activation, which converts GTP to cGMP [7]. In turn, cGMP causes protein kinase G (PKG) to activate myosin light chain (MLC) phosphatase, preventing vasoconstriction and promoting vasodilation.

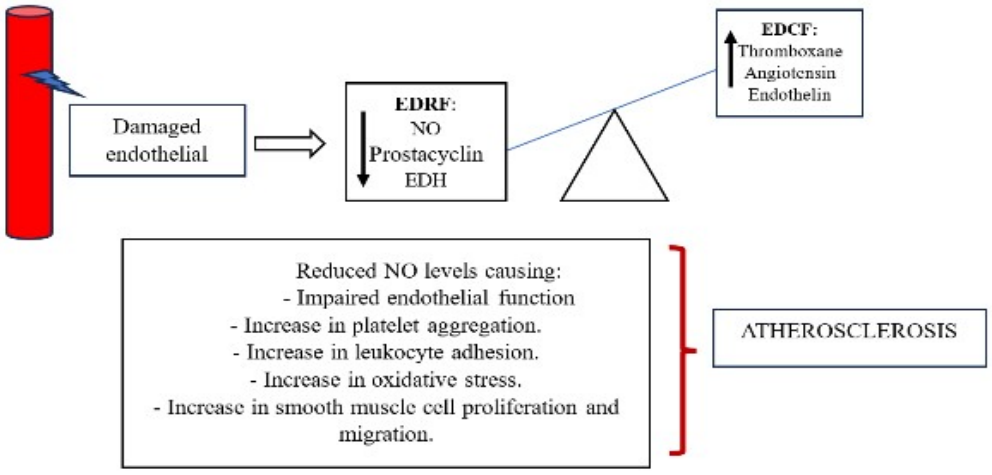

Figure 1: Effect of reduced NO bioavailability in vessels of damaged vessel. In healthy blood vessels, endothelium-derived relaxation factor (EDRF) mediates relaxation of blood vessels. However, in damaged vessels, EDRF formation is hampered by a progressive increase in endothelium contracting factor (EDCF). This imbalance, particularly decrease in NO production, leads to endothelial dysfunction, increased platelet aggregation, continuous smooth muscle cell proliferation and migration, increased leukocyte adhesion and elevated oxidative stress contributing to atherosclerosis

In VSMC, cGMP induces PKG to activate myosin light chain phosphatase, which prevents MLC from binding to actin, thereby preventing vasoconstriction and promoting vasodilation [8]. Also, PKG opens large conductance calcium-activated potassium channels (BKCa channel), which results in hyperpolarization of cell membrane and closure of voltage-dependent Ca^{2+} channels. This causes a decrease in intracellular Ca^{2+} concentration, leading to relaxation [9]. Protein kinase G further promotes vasorelaxation by phosphorylating both inositol triphosphate receptor (IP3R) and IP3R-associated PKG-I substrate (IRAG), inhibiting calcium release from endoplasmic reticulum [10]. A cGMP-dependent protein kinase activation usually causes NO-mediated relaxation, but NO directly activates sarcoendoplasmic reticulum calcium ATPase (SERCA), leading to the removal of Ca^{2+} from smooth muscle cytosol into the sarcoplasmic reticulum (Figure 2). Additionally, cGMP lessens Ca^{2+} release from the sarcoplasmic reticulum and aids in restoring Ca^{2+} to the sarcoplasmic reticulum [11].

Impairment in NO-mediated relaxation in diabetes

Reactive oxide generation

Studies have shown that a decrease in NO insufficiency contributes to impairment of NO-mediated relaxation in both human [4] and experimental settings [12]. Endothelial nitric oxide synthase typically generates NO which promotes vasodilation. However, in some diseases, the synthesis of eNOS is impaired, leading to a lack of NO production. Studies have shown that eNOS activity may increase in these cases, but instead of producing NO, it generates superoxide, a phenomenon referred to as eNOS uncoupling [13]. In diabetes, the impairment of NO-mediated relaxation is caused by various factors. Hyperglycemia in diabetes results in the production of reactive oxygen species (ROS) including hydroxyl radical (OH), superoxide anion (O_2^-), H_2O_2, lipid peroxides and hypochlorous acid (HClO). The increased levels of ROS disrupt the balance between vasodilators and vasoconstrictors.

The enzyme, NADPH oxidase (NOX) and mitochondrial electron transport chain (mETC) pathway are other sources of ROS that lead to atherosclerosis [14]. NOX triggers eNOS uncoupling, xanthine oxidase activity and mitochondrial enzymes to produce more ROS [15]. Upregulation in NADPH oxidase activity has been noticed in coronary and peripheral arteries of coronary artery disease patients [16] suggesting that NOX activation contributes to CVD development. There are five isoforms of NOX (NOX1, NOX2, NOX3, NOX4 and NOX5) are expressed in VSMC whereas NOX2 and NOX4 are found in the endothelium.

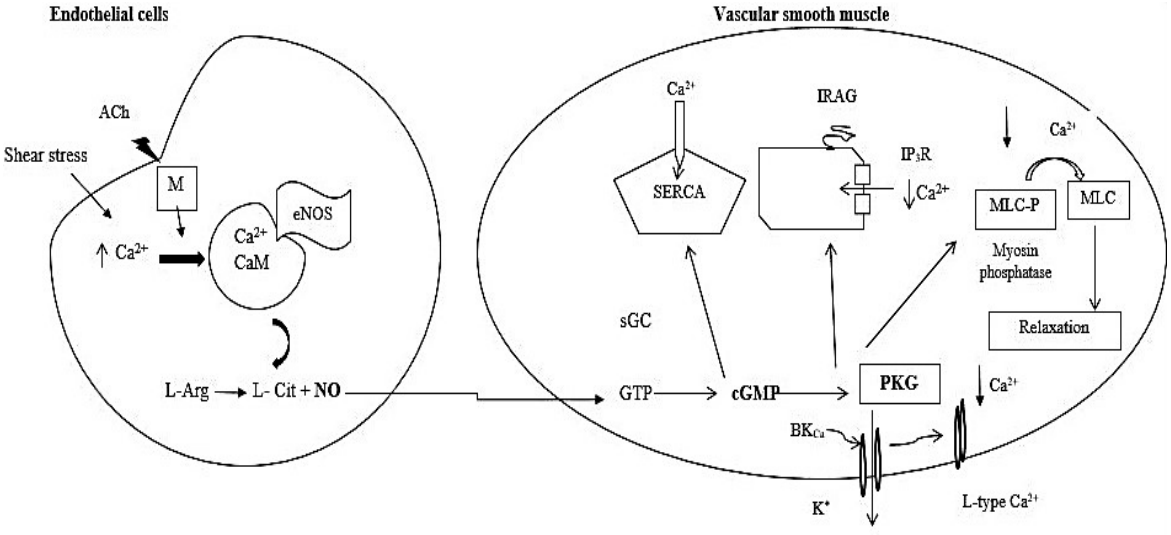

Figure 2: Diagram showing how NO causes blood vessel relaxation. Endothelial cells, when activated by shear stress or ACh, increase intracellular Ca^{2+}, triggering NO production from L-arginine by eNOS. NO diffuses to vascular smooth muscle, activating sGC to convert GTP to cGMP. This activates PKG, leading to relaxation through a) activating myosin phosphatase to prevent myosin-actin binding; b) phosphorylating BKCa channels to hyperpolarize smooth muscle cells and close L-type Ca^{2+} channels, reducing Ca^{2+} influx; and c) phosphorylating IRAG to reduce Ca^{2+} release from SERCA into VSMC cytosol. NO directly targets the SERCA to promote the removal of Ca^{2+} from smooth muscle cell

Among these isoforms, NOX4 releases hydrogen peroxide (H_2O_2), exhibiting atheroprotective effects by preventing peroxynitrite generation [17]. The release of H_2O_2 by NOX4 lowers VSMC proliferation, prevents vascular remodeling and inflammation and maintains eNOS under vascular stress [18]. Although NOX4 has a protective role, it has demonstrated detrimental effects on vascular health in several animal models depending on the NOX enzyme expressed-cell type, amounts of the released compound and its subcellular location [14].

Use of NADPH inhibitors such as apocynin, salvianic acid A, GKT136901, GKT137831, tempol and gp91dstat was shown to slow down atherosclerosis progression [14]. Angiotensin receptor inhibitors and statins used to treat human disorders are shown to inhibit NOX activity both physiologically and pathologically [14]. It has also been shown that dietary nitrate significantly boosts the production of vascular NO mainly by inhibiting vascular NOX and oxidative stress in $ApoE^{-/-}$ mice [19].

Another source of ROS is xanthine oxidase, which triggers oxidation of hypoxanthine and xanthine to produce superoxide anion and H_2O_2 [20]. Treatment with allopurinol decreases oxidative stress and improves endothelial function [21]. Reactive oxygen species may also be generated by the mitochondrial respiratory electron transport chain (ETC), another significant source of harmful ROS, leading to complications such as atherosclerosis and coronary heart disease [22]. Mitochondria are central organelles responsible for producing ATP through cellular respiration. High blood glucose environment causes impairment in electron transport chain resulting in proton leakage which leads to excessive ROS production [23].

To correct mitochondrial dysfunction and suppress atherosclerosis progression, a few therapeutic interventions are used which include antioxidants and mitochondrial inhibitors. Use of antioxidants derived from plants such as resveratrol, salidroside, ilexgenin A, berberine, quercetin, vitexin, baicalin and crocin are found to inhibit ROS production in mitochondrial cells and thus prevent the occurrence of atherosclerosis [24]. Various therapeutic mitochondrial inhibitors have been synthesized to correct mitochondrial dysfunction, including mito-TEMPO, mitochondrial fusion (M-hydrazone) and fission (MDIVI-1 and P110) [24]. Coenzyme Q_{10}, a component of mitochondrial respiratory chain, improves mitochondrial function by reducing oxidative stress. Pioglitazone and rosuvastatin, commonly used anti-diabetic and antihyperlipidemic drugs, respectively, ameliorate atherosclerosis by inhibiting mitochondrial ROS activation [24].

Role of nitrite and nitroxyl (HNO)

Besides eNOS, NO is also produced from nitrite and dietary nitrate. A recent study involving both humans and mice has shown that supplementing aging individuals with inorganic nitrite improves endothelial function by increasing NO, decreasing mitochondrial ROS/oxidative stress and increasing mitochondrial stress resistance [25]. Similarly, a reduced form of NO called nitroxyl (HNO) regulates endothelium-dependent relaxation [26,27]. However multiple studies showed that endothelium-dependent relaxation by HNO was preserved or enhanced in diabetes and not susceptible to superoxide anions ($\cdot O_2^-$) [26,28]. This preserved vasodilation of HNO could be due to lack of reactivity of HNO with $\bullet O_2$ to form ONOO [26,29]. Studies also showed that HNO directly inhibits vascular NADPH oxidase, which is responsible for superoxide production [30]. Some studies reported that HNO is a by-product of eNOS produced when L-arginine is converted to NO. It has also been suggested that eNOS produces HNO in higher quantities than NO due to eNOS uncoupling and tetrahydrobiopterin (BH_4) deficiency [31,32]. This unique property of HNO, which resists oxidative stress, may be explored as a potential therapeutic strategy for treating vascular dysfunction.

Role of endoplasmic reticulum stress

Apart from ROS, endoplasmic reticulum (ER) stress causes macro- and microvascular complications in diabetes. In hyperglycemia, ER equilibrium is interrupted leading to misfolding or unfolding of proteins and therefore causing ER stress. In response to ER stress, the unfolded protein response (UPR) signaling network is triggered to fix normal ER function. This leads to the upregulation of chaperone expression, which assists in folding ER proteins. However, this signaling network may become harmful by triggering inflammatory responses and increasing reactive oxidative stress [33,34]. Additionally, it affects endothelial function by downregulating eNOS, which also affects NO production [34].

In animal studies, impairment in endothelium-dependent relaxation was evident with the use of ER stress inducer, tunicamycin [35]. In addition, use of an ER inhibitor, tauro-ursodeoxycholic acid (TUDCA) was seen to improve vascular function, associated with normalization of myogenic response in spontaneously

hypertensive rats [35] and type 2 diabetic rats [36]. Combination treatment of TUDCA and 3′,4′-dihydroxyflavonole (DiOHF) were seen to restore endothelium-dependent relaxation and expression of eNOS, which was impaired with TUDCA treatment [37].

Another drug, metformin, was also found to reduce ER stress in angiotensin II (Ang II)-induced hypertension. In this study, mice infused with angiotensin II showed a rise in blood pressure related to enhanced vascular ER stress markers. Metformin acts as a potent activator of AMPK that reduces ER stress and therefore preserves vascular function in hypertension [38].

Role of asymmetric dimethylarginine (ADMA) and advanced glycation end products (AGEs)

Elevated blood sugar levels increase the production of asymmetric dimethylarginine (ADMA), which is known to inhibit eNOS, and lead to endothelial dysfunction (ED). This inhibition makes the ratio of arginine to ADMA a key indicator of NO availability and the risk of atherosclerotic plaque formation [39]. Increased ADMA also elevates oxidative stress possibly caused by reduced activity of dimethylarginine dimethylaminohydrolase (DDAH), the enzyme that breaks down ADMA [40]. Nitrosylation of DDAH enzymes due to increased nitrosative stress further increases ADMA concentration [41]. Production of advanced glycation end products (AGEs) is amplified in diabetes, which correlates with NO insufficiency [42]. They bind their respective receptors (RAGE), activate NF-κB and cause upregulation of vascular endothelial growth factor (VEGF), vascular cell adhesion molecule-1 and inflammatory products, which possess a significant risk factor for vascular dysfunction [43]. Accumulation of AGEs crosslinks with extracellular proteins, altering the properties of structural proteins. This disruption compromises the integrity of vascular structure, ultimately resulting in vascular stiffening and myocardial dysfunction [44]. A recent study found that patients with established diabetes had higher levels of serum AGEs and intima-media thickness of common carotid arteries compared to those with newly diagnosed diabetes, indicating greater risk factors for CVD [45]. Serum AGEs levels were found to be positively correlated with plasma ADMA levels. Advanced glycation end products (AGEs) suppress DDAH-II, an enzyme responsible for ADMA degradation, thereby reducing its total enzymatic activity and resulting in increased ADMA levels [46]. Drugs that are specifically designed to halt AGE formation, split cross-links, or inhibit AGE receptors are used clinically. Cross-link breakers are drugs that disrupt cross-links between AGEs and extracellular molecules. They include thiazolium derivatives, like N-phenacyl thiazolium bromide (PTB) and alagebrium or ALT-711, and pyridinium derivatives, like TRC4186 and TRC4149. Inhibitors of AGE, such as aminoguanidine, pimagedine, carnosine, benfotiamine, and pyridoxamine, block the formation of AGE cross-links, thereby reducing AGEs. On the other hand, RAGE antagonists such as sRAGE, RAGE antibodies and RAGE inhibitors (Azeliragon) are also used to control RAGE activation as they trigger inflammation and cause general cell dysfunction [47]. Notably, antidiabetic drugs such as metformin, thiazolidinediones, meglitinides, sulfonylureas and dipeptidyl peptidase 4 inhibitors, lipid-lowering drugs like statins, and antihypertensive agents such as angiotensin receptor blockers, angiotensin-converting enzyme inhibitors and calcium channel blockers, are currently used as these drugs inhibits AGEs production [47].

Excessive accumulation of peroxynitrite causes damage to natural antioxidants such as reduced glutathione (GSH) and superoxide dismutase (SOD) [48,49]. Peroxynitrite is responsible for direct oxidation of GSH and inactivation of SOD through nitration [50]. This leads to diminished cell antioxidant defense mechanisms, increasing superoxide levels.

Role of diacylglycerol (DAG) and protein kinase C pathway

It is important to highlight that elevated blood glucose levels, or hyperglycemia, lead to an increased production of diacylglycerol (DAG), which subsequently triggers activation of the protein kinase C (PKC) pathway [51]. As a result of increased oxidative stress, activation of PKC stimulates the production of NADPH oxidase and AGEs, both of which are widely recognized as contributors to ED [52].

Role of hexosamine pathway and protein O-GlcNAcylation

Hyperglycemia, through hexosamine pathway activation, increases protein O-GlcNAcylation which causes impairment in NO-mediated relaxation. This is linked with decreased phosphorylation of eNOS by Akt where O-GlcNAc competitively inhibits binding site of eNOS phosphorylation [53]. Masaki *et al*. reported that protein O-GlcNAcylation changes phenotype of endothelial cells in people with diabetes [54]. Protein O-GlcNAcylation was found to augment oxidative stress through activation of NADPH oxidase, which impairs

vascular function [55]. The use of glutamine inhibitors that inhibit the hexosamine pathway reversed the suppression of eNOS phosphorylation [53] and reinstated endothelium-dependent arterial relaxation in diabetic rats. Overexpression of O-GlcNAcase (OGA) that mediates removal of protein O-GlcNAcylation was found to improve vascular relaxation in a diabetic mouse model, implying that inhibiting O-GlcNAc restores endothelial function impaired by hyperglycemia [55].

Glycocalyx disruptions

Hyperglycaemic conditions disrupt glycocalyx (GCX) *in vivo* and *in vitro*. Glycocalyx downregulates eNOS expression and therefore reduces the production of nitric oxide. Recent studies suggest that empagliflozin, an SGLT2 inhibitor used to treat type 2 diabetes, decreases inflammation in endothelium and alleviates endoplasmic reticulum stress caused by prolonged glycocalyx disruption [56]. Empagliflozin also reduced oxidative stress and improved diabetes-induced vascular dysfunction in streptozotocin-induced diabetic rats [57]. The anesthetic, sevoflurane, was seen to promote endothelial glycocalyx restoration and vasodilation by increasing sialyltransferase expression during oxidative stress [58].

CONCLUSION

Impairment in endothelium-dependent relaxation is noticed in diabetes, where endothelial function is primarily regulated by NO. Increased oxidative stress and activation of several pathways contribute to impaired relaxation and ED in diabetes. Therefore, understanding the role of NO and the pathophysiology of disordered endothelial responses in diabetes will aid in selecting the appropriate therapeutic agents needed to reinstate endothelial function and prevent vascular complications of diabetes.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Seetha Munisamy analyzed and wrote the manuscript, Hemaniswarri Dewi Dewadas proofread and wrote the manuscript, Uma Eswari Punchanathan proofread the manuscript, Vaughn Alexei Ng Bansing, Lucas Cashev, Kavinash Selva Ganesan collected data.

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