REVIEW

Post-translational modifications of endothelial nitric oxide synthase induced by oxidative stress in vascular diseases

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Abstract

Endothelial nitric oxide (NO) plays a critical role in vascular homeostasis. It regulates the vascular tone, maintains the blood flow, exerts a vasorelaxing effect on smooth muscle cells and inhibits their proliferation. In the endothelium, NO is synthesized by the endothelial NO synthase which is activated by dimerization in the presence of L-arginine (as substrate) and several cofactors including the essential cofactor tetrahydrobiopterin. The NO/eNOS pathway is basically vasculoprotective and antiatherogenic but it may become dysfunctional and proatherogenic under conditions that locally increase the production of reactive oxygen species, leading to tetrahydrobiopterin oxidation, eNOS dysfunction and uncoupling. In these conditions, eNOS synthase switches from a NO-producing to superoxide anion-producing enzyme, which potentiates oxidative and nitrosative stress via the generation of peroxynitrite. In redox-perturbed conditions, eNOS dysfunction may also result from post-translational modifications deriving from oxidative stress such as S-glutathionylation or resulting either from the formation of adducts on eNOS by lipid-oxidation-derived aldehydes or from hyperglycemia-induced modifications. In this review, we summarize the mechanisms by which these post-translational modifications alter eNOS activity, and their potential implication in the pathophysiology of vascular diseases.

Introduction

Nitric oxide (NO), ‘molecule of the year 1992’ (Culotta & Koshland 1992) and ‘1998 Nobel Prize in Physiology or Medicine’ (1998 Nobel Prize in Physiology, Drs. RF Furchgott, LJ Ignarro and F Murad), is a highly reactive gaseous agent and a free radical (NO°, most of the time written NO). It is synthesized from L-arginine by NO synthases (Knowles & Moncada 1994). NO is able to penetrate through cell membranes, transmit information between cells and regulate cell functions. It is implicated in a huge variety of physiological and pathophysiological processes, spanning from cardiovascular, immune and nervous systems (for recent review, see Farah et al. 2018). In endothelial cells, NO (formerly endothelial-derived relaxing factor) is synthesized by the endothelial NO synthase (eNOS, NOS3), one of the three NO synthase isoforms, which is constitutively expressed in endothelium (Moncada & Higgs 2006).

NO exerts essential physiological functions in vascular homeostasis including the regulation of the vascular tone, blood flow and vessel vasodilation. NO may diffuse...
to adjacent cells to promote smooth muscle cell (SMC) relaxation and inhibit their proliferation (Fig. 1). NO and NO-derived species such as S-nitroso-glutathione (GSNO) may bind to cysteine residues of proteins and elicit their S-nitrosylation (Erwin et al. 2005, Heiss & Dirsch 2014). By this mechanism, the S-nitrosylation of haemoglobin-α is a source of NO in hypoxic tissues and is involved in SMC relaxation (Farah et al. 2018). NO promotes angiogenesis and contributes to upregulate the expression of the vascular endothelial growth factor (VEGF) in coordination with the hypoxia-inducible factor 1-alpha (HIF-1α) and heme-oxygenase-1 (Chen & Zheng 2014). NO may diffuse in the vessel lumen and exert antiaggregant and antithrombotic functions (Farah et al. 2018). In placenta, eNOS is expressed in cytotrophoblasts and in syncytiotrophoblasts (Eis et al. 1995). It plays a key role in the early steps of placentation, in the vasodilation of spiral arteries, blood pressure regulation, placentation and VEGF synthesis (Zullino et al. 2018, Sutton et al. 2020).

The NO/eNOS system exerts a dual role in the pathophysiology of cardiovascular diseases (CVD). This system is basically antiatherogenic and vascular protective but becomes dysfunctional and proatherogenic under conditions that locally increase the production of reactive oxygen species (ROS), resulting in eNOS dysfunction and uncoupling. In these conditions, eNOS switches from a NO-producing to superoxide anion-producing enzyme, which potentiates oxidative and nitrosative stress via the interaction of NO with superoxide anion \( \text{O}_2^- \), generating in turn peroxynitrite (ONOO⁻) and decreasing NO availability (Gimbrone & García-Cardeña 2016, Förstermann et al. 2017). The dysfunction of eNOS and its uncoupling is the main cause of endothelium dysfunction and an early event in atherosclerosis and CVD, affecting the antihypertensive, antithrombotic and antiatherogenic properties of the endothelium (Förstermann & Sessa 2012, Gimbrone & García-Cardeña 2016, Förstermann et al. 2017). During pregnancy, oxidative stress and eNOS dysfunction are the causes of poor placentation due to the decreased NO bioavailability which affects cytotrophoblast migration, spiral artery remodelling and the maintenance of blood flow (Osol et al. 2017, Zullino et al. 2018, Sutton et al. 2020).

Oxidative stress is involved in the NO/eNOS dysfunction in the endothelium, either via the oxidation and decreased availability of eNOS substrates and co-factors or via oxidative stress-induced post-translational modifications including S-glutathionylation, that is, post-translational modification by oxidized glutathione (GSGG) of cysteines involved in the physiological eNOS function (Zweier et al. 2011). Oxidative stress may also promote the formation of lipid peroxidation products and reactive carbonyl compounds, which form adducts on eNOS and inhibit its activity (Guerby et al. 2021).

This review summarizes the mechanisms by which these post-translational modifications alter eNOS activity and their possible implication in the pathophysiology of CVD.

**The NO/eNOS signalling pathway**

NO is produced by NOS which catalyse the oxidation of \( \text{l-arginine} \) to form NO and \( \text{l-citrulline} \). Three isoforms of NOS are identified in mammalian cells, the neuronal (nNOS), the inducible (iNOS) and the endothelial NOS (eNOS, NOS3). eNOS is present in endothelial cells throughout the macro and microvasculature (Heaps et al. 2019), in placental vessels and in syncytiotrophoblasts (Conrad et al. 1993, Garcia & Sessa 2019). All NOS function...
in a homodimeric state, each monomer containing an oxygenase domain in the N-terminal section with binding sites for the heme group (the catalytic site of NOS), the cofactor tetrahydrobiopterin (BH4) and the substrate L-arginine, and a reductase domain in the C-terminal section, with binding sites for NADPH and FAD (flavin adenine dinucleotide) (Förstermann & Sessa 2012, Garcia & Sessa 2019). Both oxygenase and reductase domains are linked by an interacting ‘central linker’ or calmodulin (CaM)-binding domain (Alderton et al. 2001, Aoyagi et al. 2003). Both eNOS and nNOS are activated by the Ca2+/CaM binding, whereas the inducible iNOS expressed in macrophages in response to inflammatory agents is not Ca2+-dependent (Aoyagi et al. 2003). The Ca2+/CaM binding to the ‘central linker’ promotes the transfer of electrons from NADPH through FAD and FMN (flavin mononucleotide) to the heme group of the opposite dimer (Aoyagi et al. 2003).

(6R)-5,6,7,8-tetrahydro-1-biopterin (tetrahydrobiopterin, BH4) (Crabtree & Channon 2011) is an essential cofactor for NOS activity, which facilitates electron transfer from the reductase to the oxygenase domains, maintains the heme group in its redox-active form, promotes the formation of active NOS homodimers (eNOS coupling) and prevents the release of O2•− from the eNOS oxygenase domain (Alp et al. 2003). BH4 binds to the interface of the two monomers where it is directly involved in the L-arginine oxidation process by supplying an electron to heme. In the absence or loss of BH4, L-arginine cannot bind to its site, NOS becomes uncoupled and produces O2•− rather than NO (NOS uncoupling) (Crabtree & Channon 2011, Luo et al. 2014).

Once formed, NO rapidly diffuses across cell membranes and activates the soluble guanylate cyclase (sGC), which catalyses the conversion of GTP to cyclic GMP (cGMP). cGMP is responsible for the NO vasorelaxing properties by activating the protein kinase G (PKG), which inhibits the release of Ca2+ from intracellular stores and promotes SMC relaxation (Horowitz et al. 1996). The NO/PKG pathway regulates SMC proliferation, endothelial cell permeability, motility, platelet adhesion and aggregation and thrombosis (d’Ascenzo et al. 2002, Tziros & Freedman 2006). NO decreases the activity of the Rho-like small GTPase Rac1 and inhibits the activation of the NADPH oxidase 2 (NOX2) and the production of O2•− (Brandes et al. 2014).

**Physiological regulation of eNOS activity by post-translational modifications**

The physiological activity of eNOS is regulated by the bioavailability of BH4 and arginine, by the redox environment and by post-translational modifications such as phosphorylation, S-nitrosylation, acetylation or acylation (for review see Heiss & coll) (Heiss & Dirsch 2014). In pathological conditions associated with increased ROS production and oxidative stress, eNOS may undergo post-translational modifications by S-glutathionylation or by reactive carbonyl compounds, which alter its activity, promote its uncoupling, potentiate oxidative stress and decrease NO bioavailability (Guerby et al. 2021).

**Acylation**

The acylation of eNOS (N-myristoylation and thiopalmitoylation) regulates its presence in the lipid-rich environment of caveolae. In resting cells, eNOS interacts with caveolin-1 which reversibly inhibits its enzymatic activity (Mineo & Shaul 2012). Many signalling agents can activate eNOS in caveolae via receptor-dependent systems associated with an increase in intracellular Ca2+ (lipoproteins, growth factors, bradykinin, acetylcholine...) or via receptor-independent pathways such as calcium ionophores (Kuchan & Frangos 1994, Sessa et al. 2004, Gimbrone & García-Cardena 2016). Upon eNOS stimulation, the binding of the Ca2+/CaM displaces eNOS from caveolin-1 (Balligand et al. 2009, Mineo & Shaul 2012). It is to note that caveolae interact with the cytoskeleton and undergo deformation/reorganization in response to stretching and physical stimuli, which may explain how shear stress modulates the expression and activation of eNOS present in caveolae (Lisanti et al. 1994, Balligand et al. 2009, Heiss & Dirsch 2014).

**Phosphorylation**

The site-specific phosphorylation of several serine/threonine epitopes represents a main (positive or negative) regulatory mechanism of eNOS activity (Heiss & Dirsch 2014). The positive regulation of eNOS activity mainly requires its phosphorylation at serine 1177 (Ser 1177). It is catalysed by several kinases, particularly by Akt through a PI3Kinase-dependent mechanism in response to shear stress, growth factors or ROS (Dimmel er et al. 1999, Heiss & Dirsch 2014). Interestingly, hydrogen peroxide (H2O2) generated from the dismutation of O2•− by the Cu/Zn superoxide dismutase 1 (SOD1) stimulates eNOS expression (Drummond et al. 2000) and promotes its phosphorylation on Ser 1177 through the PI3K/Akt pathway (Thomas et al. 2002). The phosphorylation of Ser 1177 is also promoted by AMPK (AMP-activated protein kinase) in response to nutrient starvation, adiponectin or salicylate (Chen et al. 2003, Hawley et al. 2012, Heiss & Dirsch 2014). In contrast,
threonine 495 (Thr 495) negatively regulates eNOS activity by interfering with the CaM binding (Heiss & Dirsch 2014). Upon eNOS stimulation, Thr 495 is dephosphorylated (mainly) by protein phosphatase PP1 and PP2A (Fleming et al. 2001, Heiss & Dirsch 2014).

Acetylation
eNOS is constitutively acetylated at two lysine residues, Lys 496 and Lys 506, in its CaM-binding domain, which inhibits its activity. The histone deacetylase SIRT1 directly activates eNOS through deacetylation and increases the bioavailability of NO (Mattagajasingh et al. 2007). Aspirin (acetyl-salicylic acid) could promote eNOS acetylation on Lys 610, which positively activates its activity by promoting the binding of CaM (Jung et al. 2010).

S-nitrosylation
S-nitrosylation of eNOS depends on its acylation and subcellular location in caveolae at the plasma membrane. Animals with eNOS mutated on acylation sites do not undergo S-nitrosylation (Erwin et al. 2005). S-nitrosylation affects several cysteine residues on eNOS and (reversibly) inhibits eNOS activity (Erwin et al. 2005, 2006). The S-nitrosylation of two cysteine residues, Cys 94 and Cys 99, has been particularly investigated since these cysteine residues form the zinc-tetrathiolate complex at the eNOS dimer interface and are involved in eNOS dimerization, which could be impaired by S-nitrosylation (Erwin et al. 2005, 2006).

Oxidative stress and ONOO− trigger eNOS uncoupling in CVD
In vascular cells and tissues, redox homeostasis is physiologically regulated by the balance between ROS-producing systems (NADPH oxides, the mitochondrial respiratory chain and xanthine oxidase or cytochrome P450) and neutralizing or antioxidant (enzymatic and non-enzymatic) systems (Forrester et al. 2018). Aging and pathological conditions including disturbed oscillatory flow, atherosclerosis risk factors (diabetes, hypertension, dyslipidemia, obesity and metabolic syndrome), pathological pregnancies, inflammatory and neuro degenerative diseases are associated with an increased production of ROS (Hulsmans & Holvoet 2010, Singh et al. 2015, Förstermann et al. 2017, Tenório et al. 2019).

Most cardiovascular, genetic and environmental risk factors, dietary habits, cigarette smoking, as well as atherogenic stimuli (angiotensin-II, mechanical forces of shear stress, oxidized LDL, cytokines…), promote endothelial dysfunction, by increasing concomitantly the expression and activation of eNOS (and NO production) and ROS-producing systems such as NADPH oxidase (NOX2) which generates O2− (Hulsmans & Holvoet 2010, Singh et al. 2015, Förstermann et al. 2017, Yuan et al. 2019). NO reacts with O2− to form peroxynitrite ONOO− (Beckman & Koppenol 1996, Radi et al. 2004), a powerful prooxidant, cytotoxic, proinflammatory and nitrating agent, involved in the ‘nitrosative stress’ (Pérez-Torres et al. 2020). ONOO− alters NO signalling by inhibiting the activity of sGC and the production of cGMP in aortic rings of hypercholesterolemic rat, inhibiting in turn the endothelium-dependent vasorelaxation and reducing intracellular cGMP levels (Francois & Kojda 2004, Korkmaz et al. 2013). In physiological conditions, NO availability is higher than that of ONOO−, so there are no consequences for vascular homeostasis. In contrast, in pathological conditions, there is an overproduction of ROS leading to an imbalance of the NO/ONOO− ratio, promoting a decreased NO bioavailability and eNOS uncoupling which produces O2− instead of NO. Therefore, uncoupled eNOS contributes to maintain its own uncoupling, enhances oxidative stress and triggers endothelial dysfunction (Balligand et al. 2009, Luo et al. 2014).

eNOS uncoupling is characterized by a shift from the dimeric to the monomeric form of eNOS (Li et al. 2014). Different mechanisms may affect eNOS activity and trigger its uncoupling, including BH4 oxidation by ROS and ONOO−, decreased arginine availability and post-translational modifications.

BH4 oxidation
ROS and ONOO− cause eNOS uncoupling via the oxidation of BH4 to the BH3 radical, resulting in endothelial BH4 decrease and dihydrobiopterin (BH2) generation, with an increase in the BH2/BH4 ratio (Vásquez-Vivar et al. 2001, Li et al. 2014). BH2 may compete with BH4 binding to eNOS, which induces eNOS uncoupling (Schulz et al. 2008). BH2 can be reduced to BH4 by the enzyme dihydrofolate reductase, whose inhibition by siRNA reduces the bioavailability of BH4 and NO (Chalupsky & Cai 2005). Interestingly, mice mutant for the GTP-cyclohydrolase I (that is involved in BH4 biosynthesis) exhibits high levels of O2−, BH4 depletion and eNOS uncoupling in aortas (Chalupsky & Cai 2005).

Arginine depletion and ADMA
Inflammatory factors and ROS strongly stimulate the expression of arginase which degrades L-arginine to
ornithine and urea (Wu & Morris 1998, Wernly et al. 2020). ONOO− and H2O2 increase arginase-I expression, which reduces in turn the availability of arginine for eNOS (Chandra et al. 2012, Lucas et al. 2014, Luczak et al. 2020). Increased arginase activity and decreased levels of circulating arginine are observed in endothelial dysfunction (Wernly et al. 2020), and in patients affected with preeclampsia (Sankaralingam et al. 2010). Besides, the arginine protein N-methyltransferase catalyses the methylation of L-arginine into the eNOS inhibitor asymmetric dimethylarginine (ADMA), which reduces NO synthesis (Sydow & Münzel 2003, Bedford & Clarke 2004). ADMA may accumulate in redox-perturbed conditions as observed in the plasma of CVD patients (Antoniades et al. 2009, Fulton et al. 2019).

Post-translational modifications of eNOS in the pathophysiology of CVD

S-glutathionylation triggers eNOS uncoupling

S-glutathionylation is a recently described mechanism of eNOS uncoupling (Chen et al. 2010). S-glutathionylation corresponds to the modification of cysteine residues on proteins through thiol-disulphide exchange with oxidized glutathione (GSSG) or through a reaction of protein thyl radicals with reduced glutathione (GSH) (Dalle-Donne et al. 2007, Mieyal & Chock 2012). S-glutathionylation occurs spontaneously and could be accelerated by glutathione transferases, particularly the isoform GSTP1-1 (Ye et al. 2017). S-glutathionylation is reversible, the deglutathionylation process being mainly catalysed by glutaredoxins (Burns et al. 2020). Under mild oxidative stress conditions, S-glutathionylation could be an adaptive response allowing to protect redox-sensitive thiols from irreversible modifications elicited by redox fluctuations (Sánchez-Gómez et al. 2013). In pathological conditions with high oxidative stress, S-glutathionylation becomes irreversible (Dalle-Donne et al. 2007, Mieyal & Chock 2012).

S-glutathionylation strongly modulates protein activity, with gain or loss of function depending on the location of cysteine and its role in protein function (Dalle-Donne et al. 2007, Mieyal & Chock 2012, Sánchez-Gómez et al. 2013). In endothelial cells (Chen et al. 2010), S-glutathionylation could reversibly affect eNOS by modifying two cysteine residues (Cys 689 and Cys 908), located in the reductase domain of the enzyme, and involved in normal eNOS function. These modifications could be reversed by reductant agents such as dithiotreitol (Chen et al. 2010). The modification of cysteines by S-glutathionylation on eNOS leads to its uncoupling and the production of O2− (Chen et al. 2010, Zweier et al. 2011). S-glutathionylation of eNOS was observed in the vessels of hypertensive rats (and not in the vessels of normotensive animals) and was associated with a loss of endothelium-dependent relaxation (Chen et al. 2010, Zweier et al. 2011). S-glutathionylation could be involved in endothelial dysfunction evoked by angiotensin-II (Galoughi et al. 2014), hypoxia/reoxygenation (de Pascali et al. 2014) and possibly in the pathophysiology of preeclampsia (Guerby et al. 2019a).

In the early stages of pregnancy, NO contributes to the migration of cytotrophoblasts and the remodelling of uterine spiral arteries, the production of the VEGF, vasculogenesis and angiogenesis (Sutton et al. 2020). In pathological pregnancies such as preeclampsia or fetal growth restriction, there is an impairment of trophoblastic invasion leading to a poor placentation and a reduced placental perfusion. These events lead to repeated hypoxia/reoxygenation waves which promote a ‘placental oxidative stress’ characterized by antioxidant depletion, oxidative damages and inflammatory responses (Tsatsaris et al. 2008, Schoots et al. 2018, Tenório et al. 2019). Among the mechanisms involved in the pathophysiology of preeclampsia, there is a decrease in NO bioavailability associated with eNOS dysfunction (Guerby et al. 2019a, Sutton et al. 2020). Recent reports by Guerby et al. (2019b, 2021) showed that in physiological pregnancy, 40–45% of total eNOS could be S-glutathionylated at term, this probably resulting from the moderate oxidative stress persisting in placentas throughout pregnancy. In contrast in the placentas from preeclampsia, S-glutathionylation affected more than 80% of the total eNOS, which could locally decrease NO bioavailability, enhance oxidative stress and reduce the placental blood flow (Guerby et al. 2019b, 2021). These observations were confirmed by in vitro studies on cultured trophoblasts which exhibited high levels of eNOS S-glutathionylation, decreased NO production and high levels of oxidative stress upon incubation in hypoxia/reoxygenation conditions, suggesting a role for S-glutathionylation in the pathophysiology of preeclampsia and placenta-related complications (Guerby et al. 2019a, 2021).

Post-translational modifications of eNOS by reactive carbonyl compounds

Lipid oxidation-derived aldehydes

Oxidative stress promotes the oxidation of polyunsaturated fatty acids (PUFAs) and the generation of lipid...
peroxidation products such as lipid oxidation-derived aldehydes including acrolein, 4-hydroxy-2-nonenal (HNE), malondialdehyde, or 4-oxo-2-nonenal (ONE). These highly bioactive agents bind to the nucleophilic sulfhydryl and primary amine groups on proteins, to form adducts and protein crosslinks that alter their function and activity (Stadtman & Levine 2003, Forman et al. 2008, Negre-Salvayre et al. 2008).

Post-translational modifications elicit various biological effects, from hormetic responses that enhance cellular antioxidant defences at low concentrations, to inflammation, senescence or apoptosis at higher concentrations, depending on the tissue, the nature of the protein, the extent and duration of oxidative stress (Dalle-Donne et al. 2003, Chapple et al. 2013, Castro et al. 2017). We recently reported that eNOS is modified by lipid-oxidation-derived aldehydes such as ONE and at a lesser extent HNE, in the placentas of preeclamptic women (Guerby et al. 2019b). Liquid chromatography with tandem mass spectrometry studies and 3D modelling of recombinant eNOS showed that ONE could bind several lysine residues particularly K 519 located close the Ca2+/CaM-binding site and K 1085 is located close to the FAD/NADPH-binding site. Moreover, the enzymatic activity of the ONE-modified recombinant eNOS was significantly decreased, indicating that ONE-adducts may alter the interaction of eNOS with its cofactors and inhibit its enzymatic activity (Guerby et al. 2020). Though abundantly detected in preeclamptic placenta, HNE was poorly detected on eNOS even if it is actually able to bind several cysteine, histidine and lysine residues on the recombinant enzyme, but without significant consequences on eNOS activity (Guerby et al. 2020). Interestingly, ONE is not or poorly neutralized by GSH which is an efficient scavenger for HNE (Zhu et al. 2009). This could explain the accumulation of ONE-adducts on eNOS and its possible contribution to eNOS dysfunction in preeclampsia.

Hyperglycemia

Hyperglycemia could inhibit eNOS and endothelium-dependent vasorelaxation in a way depending on oxidative stress and ONOO⁻ (Brouwers et al. 2010). During diabetes, hyperglycemia stimulates oxidative stress via an overproduction of mitochondrial O₂⁻ which (among other consequences) activates the hexosamine pathway and the generation of O-linked β-N-acetylglucosamine (O-GlcNAc) on serine and threonine residues (Du et al. 2000). Hyperglycemia could trigger the formation of O-GlcNAc adducts on eNOS, more precisely on ser 1177, which is also the main Akt-phosphorylation site involved in the enzymatic activation of eNOS (Du et al. 2000). In hyperglycemic conditions, the O-GlcNAc modifications of eNOS could be correlated with a decrease in O-linked serine 1177 phosphorylation and a decreased eNOS activity. This modification could be observed in aortae from diabetic animals, suggesting its possible implication in the chronic eNOS dysfunction observed in diabetes (Du et al. 2000).

Conclusion and perspectives

In this review, we summarized the mechanisms by which oxidative or lipid oxidation-derived post-translational modifications of eNOS contribute to alter the NO/eNOS pathway (Fig. 2). An important mechanism is S-glutathionylation which triggers eNOS uncoupling with potential consequences in the pathophysiology of CVD (de Pascali et al. 2014). Preclinical studies in aortas from hypertensive rats indicated the presence of high eNOS-S-glutathionylation correlated with an alteration of the endothelium-dependent vasodilation suggesting a possible implication of this system in endothelial dysfunction (Chen et al. 2010), possibly in combination with BH4 loss, as reported in endothelial cells exposed to hypoxia/reoxygenation conditions (de Pascali et al. 2014). Likewise, high eNOS S-glutathionylation levels are observed in placentas from preeclamptic women, suggesting a possible involvement of this mechanism in the reduced NO bioavailability in the pathophysiology of preeclampsia and placental dysfunction (Guerby et al. 2020, 2021). Oxidative stress contributes to the generation of lipidoxidation-derived aldehydes via the peroxidation of PUFAs, particularly during the early steps of atherogenesis and throughout atherosclerosis (Nège-Salvayre et al. 2017). The modification of eNOS by these agents in the vascular endothelium has not been investigated so far, but could be expected in lesion-prone areas of the arterial vasculature, that is, branches, bends and curvatures that are exposed to disturbed blood flow, high oxidative stress levels and endothelium dysfunction (Kwak et al. 2014, Widmer & Lerman 2014). Endothelial dysfunction is associated with an increased permeability to LDLs which accumulate in the sub-endothelial space and undergo oxidative modifications, including the formation of lipid oxidation-derived aldehydes (Nège-Salvayre et al. 2017) which may possibly react on eNOS in endothelium and alter its activity. Likewise, hyperglycemia generates oxidative stress and O-GlcNAc modifications on the Akt-phosphorylation site on eNOS, with potential consequences in the chronic eNOS inhibition occurring in diabetes (Du et al. 2001).
These oxidative stress-related post-translational modifications of eNOS represent new therapeutic strategies to restore NO bioavailability, in combination with other therapies specifically targeting NO signalling (phosphodiesterase type 5 inhibitors, folate supplementation, arginase inhibitors, NO donors, etc.) (Farah et al. 2018). The development of thiol-reducing agents could be of interest for reversing the S-glutathionylation of eNOS, as well as endothelial dysfunction and hypertension. Chen and colleagues reported that the pretreatment by dithiotreitol of aortas from hypertensive rats abolished the S-glutathionylation of eNOS and restored the acetylcholine response of relaxation (Chen et al. 2010). Likewise, hydrogen sulphide (H₂S) therapy at low physiological concentration, could reduce oxidative stress and eNOS S-glutathionylation, increase eNOS function and restore NO levels, thereby exerting protection against endothelial dysfunction and CVD (King et al. 2014). Theoretically, dietary antioxidants should favourably modulate the vascular redox environment, eNOS function and NO production, as recently reviewed by Varadharaj et al. (2017). However, the links between the NO/eNOS pathway and ROS-producing systems are complex, with strong interconnections between oxidant and antioxidant defence systems. Therapeutic use of antioxidants or agents targeting ROS signalling or lipid peroxidation gave controversial or disappointing results in the treatment of atherosclerosis, CVD or preeclampsia. As discussed by Farah et al. (2018), it is necessary to take into account these interferences and their connections with the other mechanisms involved in eNOS regulation, to develop tissue-specific therapies allowing to target the downstream effects of NO in the vascular wall.

Declarations of interest
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Author contribution statement
Anne Negre-Salvayre (A N S) Conceived and designed the analysis, collected the data and wrote the paper. Audrey Swiader (A S) performed the analysis. Paul Guerby (P G) collected the data, contributed data or analysis tools and proofread the paper. Robert Salvayre (R S) conceived and designed the analysis, contributed data or analysis tools, wrote and proofread the paper.
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