# Roles of Vascular Oxidative Stress and Nitric Oxide in the Pathogenesis of Atherosclerosis

Ulrich Förstermann, Ning Xia, Huige Li

***Abstract:*** **Major reactive oxygen species (ROS)–producing systems in vascular wall include NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) oxidase, xanthine oxidase, the mitochondrial electron transport chain, and uncoupled endothelial nitric oxide (NO) synthase. ROS at moderate concentrations have important signaling roles under physiological conditions. Excessive or sustained ROS production, however, when exceeding the available antioxidant defense systems, leads to oxidative stress. Animal studies have provided compelling evidence demonstrating the roles of vascular oxidative stress and NO in atherosclerosis. All established cardiovascular risk factors such as hypercholesterolemia, hypertension, diabetes mellitus, and smoking enhance ROS generation and decrease endothelial NO production. Key molecular events in atherogenesis such as oxidative modification of lipoproteins and phospholipids, endothelial cell activation, and macrophage infiltration/activation are facilitated by vascular oxidative stress and inhibited by endothelial NO. Atherosclerosis develops preferentially in vascular regions with disturbed blood flow (arches, branches, and bifurcations). The fact that these sites are associated with enhanced oxidative stress and reduced endothelial NO production is a further indication for the roles of ROS and NO in atherosclerosis. Therefore, prevention of vascular oxidative stress and improvement of endothelial NO production represent reasonable therapeutic strategies in addition to the treatment of established risk factors (hypercholesterolemia, hypertension, and diabetes mellitus). (*Circ Res*. 2017;120:713-735. DOI: 10.1161/CIRCRESAHA.116.309326.)**

**Key Words:** atherosclerosis ■ nitric oxide ■ oxidative stress ■ reactive oxygen species ■ risk factors



Atherosclerosis remains a leading cause of death and disability. The formation of vascular plaques is a complex process involving the interaction of plasma lipids with the vascular wall and immune cells. Since the 1950s, oxidative modifications of lipids and proteins have been detected in vascular lesions and the degree of oxidation correlates with the severity of disease,[1] indicating a role of oxidative stress in atherogenesis.

## Role of Reactive Oxygen Species–Producing Systems in Atherosclerosis

A variety of important reactive oxygen species (ROS)–producing systems are present in the vascular wall, including NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) oxidase, xanthine oxidase, enzymes of the mitochondrial respiratory chain, and a dysfunctional, uncoupled endothelial NO synthase.[2–4] Importantly, there exists cross talk between these prooxidant systems.[5–7] NADPH oxidase can trigger endothelial NOS (eNOS) uncoupling,[8] xanthine oxidase activity,[9,10] and mitochondrial ROS production.[11–14]

### NADPH Oxidases

NADPH oxidases are expressed not only in infiltrating monocytes/macrophages but also in resident cells of the vascular wall. Consisting of 2 membrane-bound subunits (p22phox and a Nox homologue) and several cytosolic regulatory subunits, these oxidases are multisubunit enzyme complexes and produce superoxide from molecular oxygen using NADPH as the electron donor.[15,16] In contrast to Nox1 and Nox2 (that require p22phox, p47phox [or NOXO1], p67phox [or NOXA1] and Rac1[17]), Nox4 only requires p22phox and releases hydrogen peroxide instead of superoxide.[18]

Three Nox isoforms are expressed in the vascular wall of mice with Nox1[19] and Nox4[20] in vascular smooth muscle cells (VSMC), Nox2,[21] and Nox4[22,23] predominantly in endothelial cells. Recent studies have shown that Nox enzymes have differential roles in atherogenesis.[24]

Genetic deletion of Nox1 in apolipoprotein E-knockout (ApoE-KO) mice reduces atherosclerosis either on an atherogenic diet[25] or under the condition of streptozotocin-induced diabetes mellitus[26] although some controversies exist.[27] Nox1 may be especially important in diabetes mellitus–accelerated

Original received November 2, 2016; revision received December 19, 2016; accepted December 26, 2016.

From the Department of Pharmacology, Johannes Gutenberg University Medical Center, Mainz, Germany (U.F., N.X., H.L.); Center for Translational Vascular Biology (CTVB), Johannes Gutenberg University Medical Center, Mainz, Germany (H.L.); and German Center for Cardiovascular Research (DZHK), Partner Site Rhine-Main, Mainz, Germany (H.L.).

Correspondence to Ulrich Förstermann, MD, PhD, Department of Pharmacology, Johannes Gutenberg University Medical Center, Obere Zahlbacher Strasse 67, 55131 Mainz, Germany. E-mail ulrich.forstermann@uni-mainz.de



*Circulation Research* **is available at http://circres.ahajournals.org** **DOI: 10.1161/CIRCRESAHA.116.309326**

**Nonstandard Abbreviations and Acronyms**

| | |
|---|---|
| **ApoE-KO** | apolipoprotein E-knockout |
| **BH4** | tetrahydrobiopterin |
| **CAD** | coronary artery disease |
| **CCA** | common carotid artery |
| **CEP** | 2-(ω-carboxyethyl) pyrrole |
| **COX** | cyclooxygenase |
| **DKO** | double knockout |
| **eNOS** | endothelial nitric oxide synthase |
| **GPx1** | glutathione perioxidase 1 |
| **GSNO** | S-nitrosoglutathione |
| **HDL** | high-density lipoprotein |
| **iNOS** | inducible nitric oxide synthase |
| **LDL** | low-density lipoprotein |
| **nNOS** | neuronal nitric oxide synthase |
| **OSE** | oxidation-specific epitopes |
| **oxLDL** | oxidized low-density lipoprotein |
| **ROS** | reactive oxygen species |
| **SOD** | superoxide dismutase |
| **TLR** | toll-like receptor |
| **XDH** | xanthine dehydrogenase |
| **XO** | xanthine oxidase |

atherosclerosis as diabetic conditions lead to upregulation of this Nox isoform.[26,28] Nox2 is also implicated in atherogenesis[29] with some regional differences. Nox2 deficiency has no effect on atherosclerosis in the aortic sinus, but reduce atherosclerosis in the descending aorta of mice.[30,31] Consistent with the atherogenic roles of Nox1 and Nox2, deficiency of p47phx, a regulatory subunit required for the activation of the both Nox isoforms, reduces atherosclerosis in mice[32,33] (Table 1).

In contrast to the proatherosclerotic role of Nox1 and Nox2, several groups have independently shown that Nox4 protects against atherosclerosis in murine models[28,35–37] (Table 1). The protective role of Nox4 against atherosclerosis may be explained by the following facts: (1) Nox4 releases hydrogen peroxide instead of superoxide because of spontaneous superoxide dismutation within the Nox4 enzyme.[38] (2) Nox4 does not lead to peroxynitrite generation because the Nox4 product hydrogen peroxide does not interact with NO.[24,38] (3) Hydrogen peroxide produced by Nox4 maintains eNOS and heme oxygenase-1 expression in the setting of vascular stress.[18,39] (4) Nox4-derived hydrogen peroxide inhibits proliferation of vascular smooth muscle cells[40] and prevents vascular inflammation and remodeling.[28] Nevertheless, Nox4 is not universally beneficial. Detrimental roles of Nox4 have been shown in rodent models of ischemic stroke,[41,42] cardiac hypertrophy,[43] and diabetic cardiomyopathy.[44] Like other ROS, hydrogen peroxide may have both protective and damaging functions, depending on the amounts formed, the cell type expressing the Nox enzyme and its subcellular location.

Nox5 has been found upregulated in human atherosclerotic lesions,[45] in human hypertension[46] and in human diabetic nephropathy.[47] The rodent genome does not contain the Nox5 gene, making it challenging to study Nox5 in experimental settings. Interestingly, transgenic mice expressing human Nox5 in a podocyte-specific manner exhibit early onset albuminuria and elevated systolic blood pressure, suggesting a role for Nox5 in disease processes.[47] Nevertheless, the impact of Nox5 on atherosclerosis development remains elusive.[24,48]

It is notable that Nox enzymes in both monocytes/macrophages and cells of the vascular wall are required for atherogenesis. Results from bone marrow transplantation experiments using ApoE-KO and p47phox$^{-/-}$ mice indicate that NADPH oxidase activity in monocytes/macrophages is essential for low-density lipoprotein (LDL) oxidation. ROS produced by NADPH oxidases in endothelial cells and smooth muscle cells, on the other hand, are involved in endothelial activation (expression of adhesion molecules and the subsequent monocyte/macrophage infiltration) and smooth muscle cell proliferation.[49] Consistent with this concept, endothelial-specific Nox2 overexpression increases vascular superoxide production, endothelial vascular cell adhesion molecule-1 expression, and macrophage recruitment, but does not induce atherosclerosis in ApoE-KO mice.[34]

## Xanthine Oxidase

Xanthine oxidase (XO) generates superoxide and hydrogen peroxide by using molecular oxygen as an electron acceptor.[50,51] The expression and activity of endothelial XO are enhanced by proatherosclerotic stimuli such as angiotensin II treatment[10] and oscillatory shear stress.[9] In addition, XO can be released from the liver and the circulation XO adhere to endothelial cells by associating with endothelial glycosaminoglycans.[52] The activity of both endothelial XO[53] and plasma XO[52] is increased in experimental atherosclerosis, as well as in human atherosclerotic plaque,[54,55] suggesting a contribution of XO-derived superoxide to atherosclerosis. Inhibition of XO improves endothelium-dependent, NO-mediated vasodilation in aorta rings from hypercholesterolemic animals[52] and reverses endothelial dysfunction in heavy smokers.[56] XO inhibitors, such as allopurinol, tungsten,[57] and febuxostat,[51] reduce atherosclerosis development in ApoE-KO mice. However, definitive proof of its role in atherosclerosis remains to be established, as no data from genetically modified murine models are available.

## Mitochondria

Normally, mitochondrial oxidative phosphorylation generates physiological levels of superoxide, which is converted to hydrogen peroxide by the manganese-dependent superoxide dismutase (MnSOD, SOD2) and subsequently by glutathione peroxidase 1 (GPx1) to water.[58] Mitochondrial oxidative stress can occur under pathological conditions because of excessive ROS production or insufficient ROS detoxification. Indeed, atherosclerosis in human has been associated with mitochondrial oxidative stress.[59]

The importance of mitochondrial redox balance is exemplified by a global or cardiac deletion of mitochondrial SOD2, which causes perinatal lethality because of cardiac myopathy and congestive heart failure, respectively.[60,61] Heterozygous SOD2$^{+/-}$ knockout mice on ApoE-KO background show increased ROS levels in the mitochondria and accelerated atherogenesis at arterial branch points.[62]

**Table 1. Role of NADPH Oxidases in Atherosclerosis**

| Gene Altered | Genetic Background | Diet/Intervention | Atherosclerosis | References |
|---|---|---|---|---|
| *Nox1*$^{-/y}$ | ApoE-KO | Atherogenic diet | ↓ | 25 |
| *Nox1*$^{-/y}$ | ApoE-KO | Normal chow/diabetes mellitus | ↓ | 26 |
| *Nox1*$^{-/y}$ | ApoE-KO | Western diet | ↔ | 27 |
| *Nox2*$^{-/y}$ | ApoE-KO | Atherogenic diet | ↔ | 30 |
| *Nox2*$^{-/y}$ | ApoE-KO | Western diet | ↓ | 31 |
| Endothelial *Nox2* Tg | ApoE-KO | Normal chow±angiotensin II | ↔ | 34 |
| *p47phox*$^{-/-}$ | ApoE-KO | Normal chow | ↔ | 32 |
| *p47phox*$^{-/-}$ | ApoE-KO | Normal chow | ↓ | 33 |
| *p47phox*$^{-/-}$ | ApoE-KO | High fat | ↓ | 33 |
| Endothelial-*Nox4* Tg | ApoE-KO | Western diet | ↔ (12 w)<br>↓ (24 w) | 35 |
| *Nox4*$^{-/-}$ | ApoE-KO | Normal chow or high-fat diet | ↑ | 36 |
| *Nox4*$^{-/-}$ | LDL-R-KO | High-fat diet | ↑ | 37 |
| *Nox4*$^{-/-}$ | ApoE-KO | Normal chow/diabetes mellitus | ↑ | 28 |

ApoE-KO indicates apolipoprotein E-knockout; LDLR-KO, low-density lipoprotein receptor-knockout; and NADPH, reduced form of nicotinamide adenine dinucleotide phosphate.

### Uncoupled eNOS

Under physiological conditions, eNOS produces NO, which represents a key vasoprotective factor of the endothelium.[63–65] Under pathological conditions associated with oxidative stress, however, eNOS may become dysfunctional.[2,66,67] Oxidative stress contributes to endothelial dysfunction primarily because of rapid oxidative inactivation of NO by excess superoxide. In the second step, the persisting oxidative stress renders eNOS uncoupled (ie, uncoupling of $O_2$ reduction from NO synthesis), such that it produces superoxide at the expense of NO. Mechanistically, deficiency of eNOS cofactor tetrahydrobiopterin (BH4), deficiency of eNOS substrate L-arginine, and eNOS S-glutathionylation are likely to be the major causes for eNOS uncoupling.[66,68] Peroxynitrite and superoxide can oxidize BH4 leading to BH4 deficiency. ROS production from uncoupled eNOS has been shown in mouse models of atherosclerosis[69–71] and in patients with hypercholesterolemia,[72] atherosclerosis,[73] hypertension[74] or diabetes mellitus,[75] and in chronic smokers.[76]

### Cyclooxygenases

Although there is evidence that cyclooxygenases may indirectly modulate vascular ROS generation,[77] it is unlikely that the cyclooxygenase enzymes per se serve as sources of pathogenic ROS.[78] Cyclooxygenase-1 inhibition or deletion in fact attenuates atherogenesis in mice.[79,80] Experiments with cell-specific deletion of cyclooxygenase-2 in mice indicate a complex role of cyclooxygenase-2 in atherogenesis. Whereas cyclooxygenase-2 in macrophages promotes atherosclerosis development, cyclooxygenase-2 in T cells has little effect on lesion burden.[81] In contrast, cyclooxygenase-2 in endothelial cells and VSMC seems to be atheroprotective.[82] The beneficial effects of cyclooxygenase-2 have been attributed to prostacyclin biosynthesis,[82] but prostacyclin-independent mechanisms have also been proposed.[83]

## Role of Antioxidant Enzymes in Atherosclerosis

Vascular cells are equipped with a variety of antioxidant defense enzymes enabling the reduction of oxidative burden.

### Superoxide Dismutase

There are 3 SOD isoforms expressed in mammalian tissues: (1) SOD1 (copper/zinc-SOD), which is located in the cytoplasm and in the mitochondrial intermembrane space; (2) SOD2, which is expressed in the mitochondrial matrix, and (3) SOD3 (extracellular SOD), which is found in extracellular matrix, on cell surface, and in extracellular fluids.[84,85] All 3 isozymes serve key antioxidant functions by catalyzing the dismutation of superoxide into oxygen and hydrogen peroxide.[4,65]

Unexpectedly, transgenic mice overexpressing SOD1 develop more pronounced atherosclerotic lesion than control mice.[86] It is proposed that the effects of SOD on atherogenesis are dose dependent.[87] Moderate SOD1 upregulation reduces ROS burden, whereas excessive SOD activity could enhance oxidative injury by increasing formation of distal oxidants. SOD1 overexpression generates high amount of hydrogen peroxide, which can lead to the formation proatherogenic molecules such as hydroxyl radicals or metal-associated reactive species[85] (Figure 1). In supporting this concept, combined overexpression of SOD1 and catalase reduces atherosclerosis in ApoE-KO mice.[88]

SOD2 is the first line of defense against superoxide as a byproduct of the mitochondrial electron transport chain. Homozygous SOD2 mutant mice die within the first 10 days of life, indicating the importance of this enzyme.[60] SOD2 deficiency ($SOD2^{+/-}$) leads to mitochondrial dysfunction, increased mitochondrial DNA damage, and accelerated atherosclerosis in ApoE-KO mice.[62]

SOD3 is abundantly expressed in the vascular wall.[89] Paradoxically, genetic deletion of SOD3 in ApoE-KO mice leads to a slight reduction in atherosclerosis after 1-month

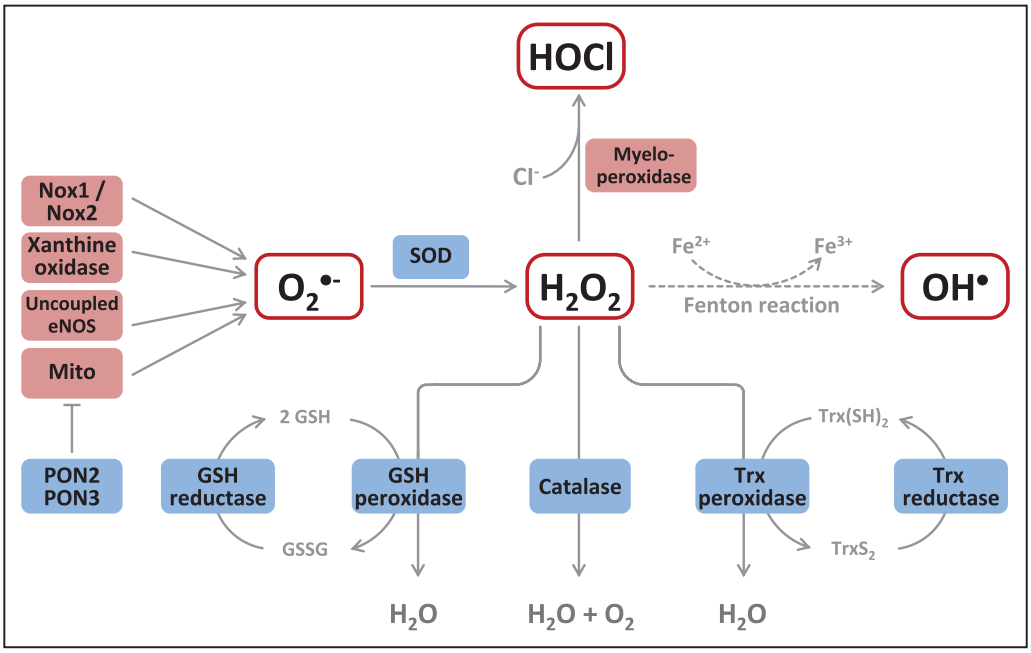


**Figure 1. Enzymes involved in the generation and inactivation of reactive oxygen species (ROS)**. Superoxide anion ($O_2^{\bullet-}$) can be produced in the vascular wall by NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) oxidases (Nox1 and Nox2), xanthine oxidase, uncoupled endothelial nitric oxide synthase (eNOS), and the mitochondrial respiration chain. $O_2^{\bullet-}$ can be converted to hydrogen peroxide ($H_2O_2$) by the enzyme superoxide dismutase (SOD). $H_2O_2$ can undergo spontaneous conversion to hydroxyl radical ($OH^{\bullet}$) via the Fenton reaction. $OH^{\bullet}$ is extremely reactive and attacks most cellular components. $H_2O_2$ can be detoxified via glutathione (GSH) peroxidase, catalase or thioredoxin (Trx) peroxidase to $H_2O$ and $O_2$. The enzyme myeloperoxidase can use $H_2O_2$ to oxidize chloride to the strong-oxidizing agent hypochlorous acid (HOCl). HOCl can chlorinate and thereby inactivate various biomolecules including lipoproteins and the eNOS substrate L-arginine. Besides HOCl generation, myelo-peroxidase can oxidize (and thus inactivate) NO to nitrite ($NO_2^-$) in the vasculature. Paraoxonase (PON) isoforms 2 and 3 can prevent mitochondrial $O_2^{\bullet-}$ generation. Reprinted from Li et al[67] with permission of the publisher. Copyright © 2013, Elsevier.

atherogenic diet, whereas no effect can be found after 3 months on the atherogenic diet or after 8 months on standard chow.[90] Therefore, functional significance of SOD3 in atherogenesis is still unclear and warrants more studies in future.

Overall, the effect of SOD enzymes on atherosclerosis is likely to be context dependent. SOD enzymes may have antiatherosclerotic effects by inhibition of oxidative damages caused by superoxide and by prevention of superoxide-mediated inactivation of NO. However, SOD may also enhance oxidative stress in case that the capacity of downstream enzymes (eg, catalase and GPx) is insufficient to detoxify SOD the product (Table 2).

## Catalase

Catalase is located exclusively in peroxisomes[58] where it catalyzes the reduction of hydrogen peroxide to oxygen and water. Overexpression of catalase reduces atherosclerosis in ApoE-KO mice.[88]

Although ROS indisputably participate in atherogenesis, it is likely that the relative contribution of different ROS varies in different atherosclerosis models (Table 2). This becomes apparent when SOD1 is compared with catalase in mouse atherosclerosis models. In ApoE-KO mice on high-fat diet, overexpression of catalase reduces atherosclerosis, whereas SOD1 overexpression is ineffective.[88,92] It is likely that superoxide makes only a minor contribution to the atherosclerosis induced by a high-fat diet or by ApoE deficiency. It has been suggested that these atherogenic stimuli (eg, oxidized low-density lipoprotein [oxLDL]) lead to the accumulation of peroxides and not superoxide.[103] In contrast, the effect of SOD1 overexpression is evident in atherosclerosis models where both hydrogen peroxide and superoxide participate in atherogenesis, such as x-ray exposure[91] or treatment with the environmental pollutant benzo(a)pyrene.[92]

## Glutathione Peroxidases

By reducing hydrogen peroxide to water and lipid hydroperoxides to their corresponding alcohols, glutathione peroxidases (GPx) represent the major antioxidant enzyme within many cells.[104] GPx1 is the ubiquitous intracellular member of the GPx family and is expressed both in the mitochondria and in the cytoplasm. A low activity of red blood cell GPx1 is associated with an increased risk of cardiovascular events in patients with coronary artery disease (CAD).[105] GPx1 deficiency increases LDL oxidation, foam cell formation, and macrophage proliferation.[106] Two independent studies have shown that deficiency of GPx1 enhances atherosclerosis in ApoE-KO mice,[94,95] indicating a protective role of GPx1 against atherogenesis. Consistently, seleno-organic GPx1-mimetics also reduce atherosclerotic lesions in diabetic ApoE-KO mice.[107]

Similar antiatherosclerotic properties have been shown for GPx4, although this GPx enzyme differs significantly from the other GPx family members in structure, intracellular localization, and functional characteristics.[108] GPx4 reduces hydrogen peroxide and a wide range of lipid hydroperoxides, including oxidized phospholipids and cholesterol hydroperoxides.[96] GPx4 overexpression reduces atherosclerosis in ApoE-KO

**Table 2. Role of Antioxidative Enzymes in Atherosclerosis**

| Enzyme | Gene Altered | Genetic Background | Diet/intervention | Atherosclerosis | References |
|---|---|---|---|---|---|
| SOD1 | *SOD1* Tg | B6 | Atherogenic diet | ↑ | 86 |
| | *SOD1* Tg | B6 | Atherogenic diet+irradiation | ↓ | 91 |
| | *SOD1* Tg | ApoE-KO | Normal chow | ↔ | 88 |
| | *SOD1* Tg+*CAT* Tg | ApoE-KO | Normal chow | ↓ | 88 |
| | *SOD1* Tg | ApoE-KO | Normal chow+BaP | ↓ | 92 |
| | *SOD1* Tg+*CAT* Tg | ApoE-KO | Normal chow+BaP | ↓↓ | 92 |
| SOD2 | *SOD2*$^{+/-}$ | ApoE-KO | Normal chow | ↑ | 62 |
| SOD3 | *SOD3*$^{-/-}$ | ApoE-KO | Normal chow | ↓ (1 mo)<br>↔ (3 mo) | 90 |
| | *SOD3*$^{-/-}$ | ApoE-KO | Atherogenic diet | ↔ (8 mo) | 90 |
| Catalase | *CAT* Tg | ApoE-KO | Normal chow | ↓ | 88 |
| | *CAT* Tg | ApoE-KO | Normal chow+BaP | ↓ | 92 |
| GPx1 | *GPx1*$^{-/-}$ | B6 | High fat | ↔ (12 wk)<br>↓ (20 wk) | 93 |
| | *GPx1*$^{-/-}$ | ApoE-KO | Western diet | ↑ | 94 |
| | *GPx1*$^{-/-}$ | ApoE-KO | Normal chow+streptozotocin | ↑ | 95 |
| GPx4 | *GPx4* Tg | ApoE-KO | Normal chow | ↓ | 96 |
| PON1 | *PON1* Tg | B6 | Atherogenic diet | ↓ | 97 |
| | *PON1* Tg | ApoE-KO | Normal chow | ↓ | 97 |
| | *PON1*$^{-/-}$ | B6 | Atherogenic diet | ↑ | 98 |
| PON2 | *PON2* Tg | ApoE-KO | Normal chow | ↓ | 99 |
| | *PON2*$^{-/-}$ | B6 | Atherogenic diet | ↑ | 100 |
| PON3 | *PON3* Tg | B6 | Atherogenic diet | ↓ | 101 |
| | *PON3* Tg | LDLR-KO | Western diet | ↓ | 101 |
| Thioredoxin-2 | Endothelial Tg | ApoE-KO | Western diet | ↓ | 102 |

ApoE-KO indicates apolipoprotein E-knockout; BaP, benzo(a)pyrene; GPx1, glutathione perioxidase 1; LDLR-KO, low-density lipoprotein receptor-knockout; PON, paraoxonase; and SOD, superoxide dismutase.

mice by preventing lipid peroxidation and oxidized lipid sensing by vascular cells.[96]

## Paraoxonases

The paraoxonase family of proteins has 3 members (paraoxonase 1, paraoxonase 2, and paraoxonase 3) with overlapping and discrete esterase and lactonase activities, metabolizing/hydrolyzing arachidonic acid oxidation products (all 3 paraoxonases), organophosphates (paraoxonase 1), quorum-sensing signals of pathogenic bacteria (N-acylhomoserine lactones by paraoxonase 2), and drugs (eg, lovastatin and spironolactone by paraoxonase 3).[109] All 3 paraoxonase proteins reduce oxidative stress, decrease lipid peroxidation, and diminish atherosclerosis, but with different mechanisms.

Paraoxonase 1 is mainly synthesized by the liver and associates with high-density lipoprotein (HDL) particles. Many of the antiatherosclerotic properties of HDL are partly attributed to the esterase, peroxidase-like, and phospholipase-like activities of paraoxonase 1.[110–112] HDL-associated paraoxonase 1 inhibits the formation of oxidized phospholipids and thus LDL oxidation. Overexpression of paraoxonase 1[97,113] reduces atherosclerosis, whereas disruption of paraoxonase 1 gene[98] exacerbates atherogenesis (Table 2).

Paraoxonase 2 is undetectable in plasma[114] but abundantly expressed in the vascular wall. Paraoxonase 2 is found in intracellular structures, such as the membranes of the ER or mitochondria, where it reduces superoxide formation and ER stress signaling.[115,116] Interestingly, paraoxonase 2 can translocate to the plasma membrane in response to oxidative stress where it suppresses lipid peroxidation and regulates glucosylceramide content.[117] Paraoxonase 2 prevents LDL peroxidation, reduces oxidative stress in all major vascular cells,[100,116] and protects against atherosclerosis in mouse models (Table 2).[99,100] In humans, paraoxonase 2 expression is found decreased in plaques versus plaque-adjacent tissue, indicating that the protective effect of paraoxonase 2 could fail during atherosclerosis development.[118]

Paraoxonase 3 is found both in serum and cells and prevents LDL oxidation like paraoxonase 1.[119] Similar to paraoxonase 2, paraoxonase 3's antioxidative effect results from the prevention of mitochondrial superoxide formation because of an interaction with coenzyme Q10 (ubiquinone).[115,120] During the Q cycle, the unstable intermediate

ubisemiquinone can donate an electron to molecular oxygen leading to superoxide production. Paraoxonase 2 and paraoxonase 3 are present in the inner mitochondrial membrane and bind to coenzyme Q10 with high affinity. The 2 paraoxonase enzymes sequester ubisemiquinone and thereby reduce mitochondrial superoxide formation.[115,120,121] Paraoxonase 3 counteracts atherosclerosis in mice,[101] and its expression level is reduced in vascular cells of atherosclerotic patients.[122]

### Thioredoxins

The thioredoxin systems involve thioredoxin, thioredoxin reductases, and thioredoxin peroxidases.[123,124] Thioredoxin can reduce target proteins via cysteine thiol-disulfide exchanges. Thioredoxin-dependent peroxidase can also directly scavenge hydrogen peroxide, and thioredoxin reductase converts oxidized thioredoxin to its reduced form to facilitate its redox activity (Figure 1). Experiments with transgenic mice have shown that both the cytosolic thioredoxin-1 and the mitochondrial thioredoxin-2 systems are essential regulators of cardiac function.[123,124] For the vascular system, compelling evidence exists supporting a crucial role of the thioredoxin-2 system. Endothelial-specific deletion of thioredoxin-R2 leads to increased vascular stiffness, impaired endothelial function, and a prothrombotic, proinflammatory vascular phenotype.[125] Endothelial-specific overexpression of mitochondrial thioredoxin-2 improves endothelial function and reduces atherosclerotic lesions in ApoE-KO mice.[102]

## Role of NO in Atherosclerosis

Results from studies using genetically modified animals indicate that NO synthase (NOS) isoforms have different roles in atherosclerosis with eNOS and neuronal nitric oxide synthase (nNOS) being atheroprotective and inducible nitric oxide synthase (iNOS) being proatherogenic.[126]

### Neuronal NO Synthase

The nNOS is expressed in not only perivascular nerve fibers but also the vascular wall[127,128] and atherosclerotic plaques.[129] Vascular nNOS contributes to vasodilation[130] and can partly compensate the loss of eNOS in eNOS$^{-/-}$ mice.[131–133] Recent human studies suggest that nNOS plays an important role in the local regulation of vascular tone independently of its effect in the central nervous system. Inhibition of nNOS reduces the basal blood flow in human forearm and coronary circulations without affecting the eNOS-mediated vasodilatation elicited by acetylcholine, substance P, or increased shear stress,[134,135] indicating distinct roles of nNOS and eNOS.

Disruption of nNOS gene enhances neointimal formation and constrictive vascular remodeling in a mouse model of carotid artery ligation.[128,136] Double knockout (DKO) mice deficient in nNOS and ApoE show markedly larger atherosclerotic lesion in the aortic root and descending thoracic aorta[129,137] and increased mortality[137] (Table 3).

### Inducible NO Synthase

The inducible isoform iNOS is normally absent in the vasculature under physiological conditions. Its expression is induced in blood vessels in pathological situations, such as inflammation, sepsis, or oxidative stress.[144]

Genetic disruption of iNOS ameliorates atherosclerotic lesion in ApoE-KO mice without changing the lipid profile[138] (Table 3). The reduced atherosclerosis in iNOS/ApoE DKO mice is associated with decreased plasma levels of lipoperoxides, indicating that peroxynitrite-mediated oxidative stress is likely to be involved in the proatherosclerotic effects of iNOS.[138]

In contrast to the regulated production of NO by nNOS and eNOS, iNOS may generate large amounts of NO over long periods of time. If induced in endothelial cells, iNOS competes with eNOS for BH4 and thus reduces eNOS-mediated NO production by limiting BH4 availability for eNOS.[145] Furthermore, iNOS induction in the vasculature facilitates the generation of peroxynitrite,[146–149] a key proatherosclerosis oxidant.[150,151] Indeed, the expression of iNOS in human atherosclerosis plaque is associated with nitrotyrosine, a marker of peroxynitrite formation.[149,152,153]

### Endothelial NO Synthase

The eNOS enzyme is constitutively expressed mainly in endothelial cells. Its activity is regulated by shear stress of the flowing blood and by agonists such as bradykinin and acetylcholine. Endothelial NO produced by eNOS induces vascular smooth muscle relaxation and inhibits platelet aggregation and adhesion.[63–65] In addition to these antihypertensive and antithrombotic properties, eNOS-derived NO also exerts multiple antiatherosclerotic effects, including inhibition of LDL oxidation, prevention of leukocyte adhesion to vascular endothelium and leukocyte migration into the vascular wall, and inhibition of vascular smooth muscle cell proliferation.[63–65] At

**Table 3. Role of NO Synthases in Atherosclerosis**

| Enzyme | Gene Altered | Genetic Background | Diet/intervention | Atherosclerosis | References |
|---|---|---|---|---|---|
| nNOS | *nNOS*$^{-/-}$ | ApoE-KO | Western diet | ↑ | 137 |
| iNOS | *iNOS*$^{-/-}$ | ApoE-KO | Western diet | ↓ | 138 |
| eNOS | *eNOS*$^{-/-}$ | ApoE-KO | Western diet | ↑ | 139–141 |
| | *eNOS*-Tg | ApoE-KO | Western diet | ↑ | 142 |
| | *eNOS*-Tg x *GCH1*-Tg | ApoE-KO | Normal chow | ↓ (vs eNOS-Tg)<br>↔ (vs ApoE-KO) | 143 |

ApoE-KO indicates apolipoprotein E-knockout; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; and nNOS, neuronal nitric oxide synthase.

low physiological levels, NO also acts as an antioxidant, abating fenton-type reactions, terminating radical chain reactions, and inhibiting peroxidases and oxidases (eg, by nitrosylation of allosteric thiols).[154]

Consistent with the antiatherosclerotic role of eNOS-derived NO, genetic disruption of eNOS in ApoE-KO mice enhances atherosclerosis[139,140] (Table 3). Unexpectedly, overexpression of eNOS also accelerates atherogenesis in ApoE-KO mice.[142] This paradoxical phenomenon is explained by later findings that eNOS overexpression leads to eNOS uncoupling owing to a relative deficiency of eNOS cofactor BH4. Indeed, reversal of eNOS uncoupling by upregulating BH4 synthesis in ApoE-KO/eNOS-Tg mice leads to a reduction of atherosclerotic lesion.[143]

The eNOS-KO mice are hypertensive. However, hypertension does not account for the accelerated atherosclerosis in the eNOS-KO animals. Treatment of ApoE/eNOS DKO mice with hydralazine lowers the blood pressure to levels seen in ApoE-KO mice, but does not change the extent of lesion formation in ApoE/eNOS-DKO mice. These results indicate that hypertension is not required for the accelerated atherosclerosis seen in apoE/eNOS DKO animals and that eNOS plays important roles in suppressing atherogenesis separate from blood pressure regulation.[140]

Recent studies have shown that eNOS is also present in the perivascular adipose tissue. Under physiological conditions, NO derived from perivascular adipose tissue–eNOS contributes to the vasoprotective effects of perivascular adipose tissue. Under pathological conditions, however, perivascular adipose tissue–eNOS may become a superoxide-generating enzyme.[155,156]

## Differences Between Murine Models and Human Pathology

Although the ApoE-KO and the LDL receptor-KO mice are excellent models for atherosclerosis research, there exist significant differences between these commonly used murine models and human pathology.[157,158] For instance, (1) these mice have usually excessive blood cholesterol levels; they are not common in human patients. (2) The majority of lipoproteins are found in the HDL fraction in mice, whereas it is in LDL and very-low-density lipoprotein in humans. (3) Murine atherosclerosis studies are mostly performed in relatively young mice without aging. (4) There are significant differences between the murine and human immune systems. (5) Mice do not develop plaque rupture on a regular basis.[157,158] Therefore, these differences must be considered when translating results from murine experiments into clinical settings.

Because of the significance of such differences, mouse models for plaque rupture have been developed. The brachiocephalic artery in ApoE-deficient mice fed a high-fat diet, with or without angiotensin II infusion, is a practically feasible model for plaque rupture.[159] It has been shown in this model that monocyte/macrophage-mediated oxidative stress and inflammation are likely to be involved in plaque destabilization and plaque rupture.[160] Recently, a mouse model with a fibrillin-1 gene mutation on the ApoE-KO background has been described as a model of acute plaque rupture with human-like complications.[161] Also in this model, lipid oxidation and iNOS-mediated inflammation are likely to play roles in the pathology.

In both the ApoE-KO and LDL receptor-KO mice, atherogenesis is driven, at least in part, by non-HDL hyperlipidemia, although the underlying mechanisms are different.[162] Because ApoE has antioxidative properties, the oxidation of lipoproteins is more prominent in apoE-KO mice than in LDL receptor-KO mice. Consistently, antibodies to oxidized LDL epitopes are especially high in the ApoE-KO mice.[162] There are no data available directly comparing the levels of endothelial NO between the 2 mouse models.

There exist sex differences in atherosclerosis,[163] both in humans[164] and mice.[165] Atherosclerotic lesion formation and lipid accumulation in the aorta from ApoE-KO mice is more pronounced in men than women.[165] These sex-dependent differences are associated with lower NADPH oxidase activity in the women.[165] Moreover, ovariectomy enhances NADPH oxidase activity, lipid deposition, and atherosclerotic lesion formation, which can be recovered by administration of 17β-estradiol, indicating the role of female sex hormones.[165,166] Furthermore, arteries from female animals also show higher expression of SOD1, SOD2, and eNOS.[167] Estrogens have been shown to enhance eNOS expression and NO production in endothelial cells.[168]

## Gene Polymorphisms and Atherosclerosis in Humans

Genome-wide association studies have identified 58 single-nucleotide polymorphisms that are genome-wide significantly associated with CAD.[169,170] Ten of the CAD loci are associated with LDL and another 5 with blood pressure,[170] which should not be surprising. On the contrary, the majority of the CAD genome-wide association study loci is not associated with known risk factors for CAD.[170] The redox genes and NO synthase genes mentioned in this article are not among the 58 CAD genome-wide association study loci, either.

In contrast to genome-wide association studies, hypothesis-driven candidate gene association studies have provided evidence for possible association of redox gene and eNOS gene polymorphisms with the risk of atherosclerosis (Table 4). Unfortunately, the results are often conflicting and inconclusive, partially because of the small sample size in each study or the differences in ethnicity.

### NADPH Oxidase Subunits

The p22phox subunit is required by Nox family members of NADPH oxidases. The Nox proteins and the p22phox protein are stable only as a heterodimer. A significant number of allelic variants have been identified within the promoter and exonic sequences of the p22phox gene.[191] Among these, particular attention has been paid to the C242T polymorphism which results in replacement of histidine by tyrosine at amino acid position 72 (H72Y), a potential heme binding site.[174] The 242T allele has been shown to be associated with reduced NADPH oxidase activity and respiratory burst in human neutrophils,[192] and with decreased vascular NADPH oxidase activity in saphenous veins of patients with CAD, independently of other

**Table 4. Gene Polymorphisms and Atherosclerosis**

| Enzyme | rs Number | Polymorphism | Functional Consequence | Effects on Atherosclerosis |
|---|---|---|---|---|
| Nox2 | rs4673 | C242T (His72Tyr) | Nox2 activity↓ | CAD ↓[171–173] |
| | | | | CAD ↑[174,175] |
| SOD1 | rs9974610 | A→G (≈13.6 kb 5' from TSS) | Unknown | CV death risk ↓[176] |
| | rs10432782 | T→G (intron 2) | Unknown | CV death risk ↑[176] |
| | rs1041740 | C→T (intron 4) | Unknown | |
| SOD2 | rs4880 | Ala16Val | ↓SOD2 into mitochondria | CAD ↑[177–179] |
| SOD3 | rs1799895 | Arg213Gly | ↓SOD3 tissue binding | CAD risk ↑[180,181] |
| GPx1 | rs1050450 | Pro198Leu | GPx1 activity↓ | IMT ↑[182]; CAD ↑[183,184] |
| | | | GPx1 activity ↔ | No effect[185,186] |
| eNOS | rs1799983 | Glu298Asp | eNOS activity↓ | CAD ↑[187–189] |
| | rs 2070744 | -T786C | eNOS expression↓ | CAD ↑[189,190] |
| | | Intron 4 VNTR | eNOS expression↓? | CAD ↑[187,189] |

CAD indicates coronary artery disease; CV, cardiovascular; GPx1, glutathione perioxidase 1; Nox2, NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) oxidase 2; SOD, superoxide dismutase; and TSS, transcription start site.

clinical risk factors.[193] Although some studies indicate that the T allele may confer protection against atherosclerosis,[171–173] some other studies have shown negative or even opposite effects.[174,175] Two recent meta-analyses have also provided conflicting results.[194,195] Therefore, more studies are needed before a definitive conclusion can be drawn.

Whereas the C242T variant is a rather common (T allele ≈20%[194]) genetic polymorphism, the chronic granulomatous disease is a rare (1 in 200 000–250 000 individuals) inherited disorder of the innate immune system and caused by genetic defects in the genes encoding 4 of the phox proteins (Nox2/gp91phox, p22phox, p47phox, and p67phox).[191] The deficiency of Nox2 activity in patients with chronic granulomatous disease results in an improved flow-mediated arterial dilation[196] and a protection of the vascular endothelium against ischemia/reperfusion-induced endothelial dysfunction in the brachial artery.[197] Moreover, individuals with chronic granulomatous disease have lower carotid intimal-medial thickness[198] and reduced carotid atherosclerosis,[199] although coronary atherosclerosis is not changed by chronic granulomatous disease.[199] All these observations support the concept that Nox2 activity contributes to atherogenesis in humans. Consistently, upregulation of Nox2 is evident in coronary arteries of human patients with atherosclerosis[55,200]

## SOD Enzymes

Association studies for SOD1 has been scarce. A recent study has found that 3 variants in the SOD gene are associated with increased risk of death from cardiovascular causes (sudden death, fatal myocardial infarction, or stroke).[176]

A functional polymorphism has been identified in the SOD2 gene resulting in the replacement of alanine 16 with a valine (Ala16Val) in the mitochondrial targeting domain. Human beings harboring this variant have an increased carotid intima-to-media thickness and are at increased risk for CAD and acute myocardial infarction.[177–179]

Genetic variants exist in both the coding region and the promoter region of SOD3 gene.[201] Most researches have concentrated on the functional variant Arg213Gly in the heparin-binding domain. The Gly allele is associated with decreased SOD3 affinity for heparin, reduced tissue binding,[202–204] and reduced antioxidant effects in the vascular wall.[204] This polymorphism has been linked to increased body weight, triglycerides, and higher cardiovascular risk[180,181] but paradoxically decreased risk of lung disease.[205]

## GPx1

The Pro198Leu polymorphism is a site located within the GPx1 C-terminal region. This amino acid substitution has been proposed to change structural conformation of the active site region and to modify the enzyme activity.[206] GPx1 polymorphism has been associated with increased carotid intima-to-media thickness, peripheral arterial disease, and increased CAD risk.[182–184,207] However, other studies found no association of Pro198Leu polymorphism with stroke[208] or coronary artery stenosis.[185,186] The predicted tertiary structure of GPx1 shows that the C-terminal fragment containing the Pro198Leu polymorphic site is located on the protein surface and within a nonfunctional fragment.[185]

## eNOS

The most intensively examined and functionally related common eNOS variants include Glu298Asp (G894T), -T786C, and the intron 4 variable number tandem repeat.[187,209] Early studies suggested that eNOS protein containing 298Asp is subject to selective proteolytic cleavage.[210,211] These observations, however, turned out to be artifacts.[212,213] A later study has provided evidence that the Glu298Asp single-nucleotide polymorphism affects eNOS localization to caveolar membrane leading to diminished shear-dependent responses and impaired coordination of the eNOS regulatory cycle.[214] A large number of genetic association studies exist addressing

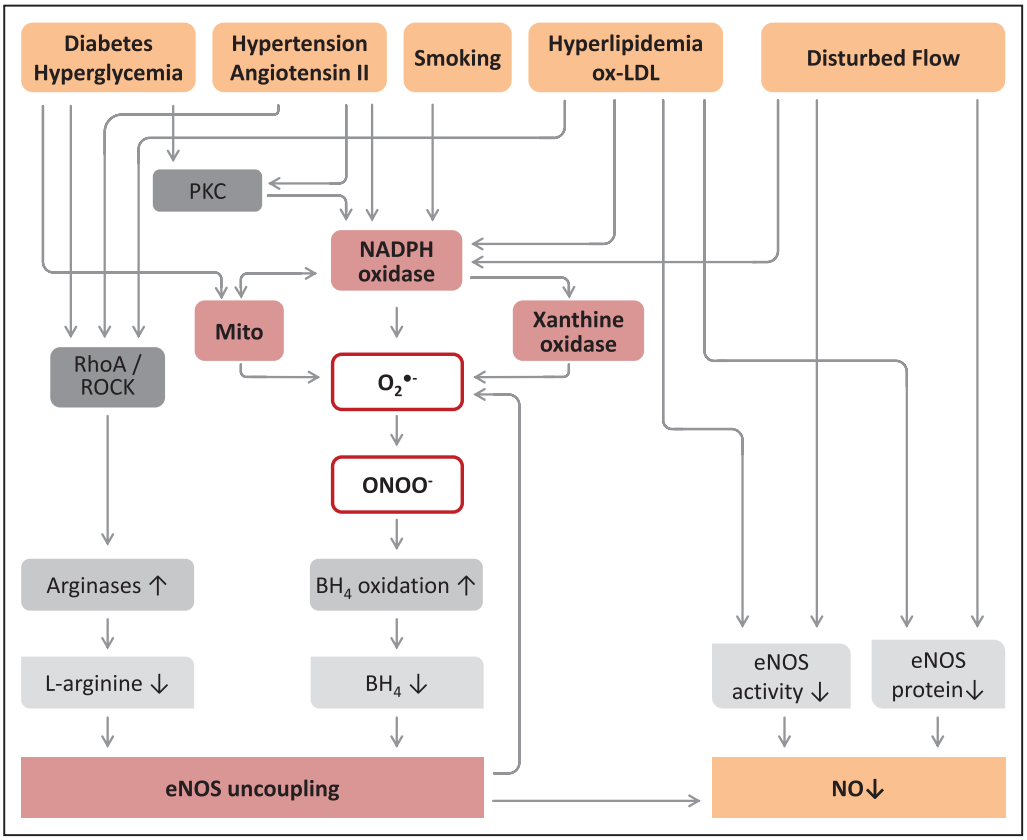


**Figure 2. Cardiovascular risk factors induce vascular oxidative stress and reduce endothelial nitric oxide (NO) production.** Hypercholesterolemia, hypertension, smoking, and diabetes mellitus lead to activation of NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) oxidase, partly through mechanism dependent on protein kinase C (PKC). Diabetes mellitus also stimulates mitochondrial ROS production, which then triggers NADPH oxidase activation. NADPH oxidase can enhance superoxide ($O_2^{\bullet-}$) production from mitochondria and xanthine oxidase. The endothelial NO synthase (eNOS) enzyme can become uncoupled through 2 major mechanisms: deficiency of the cofactor tetrahydrobiopterin (BH4) or of the substrate L-arginine. $O_2^{\bullet-}$ reacts with NO resulting in peroxynitrite ($ONOO^-$). $ONOO^-$ oxidizes BH4 leading to BH4 deficiency. L-Arginine deficiency is caused by upregulation of arginase expression and activity, partly through RhoA/ROCK-dependent mechanisms. Uncoupled eNOS produces superoxide thereby potentiating oxidative stress. The uncoupling of eNOS decreases endothelial NO production, which is further exacerbated by reduced eNOS expression and activity. In addition to the risk factors at the population level, disturbed flow renders arterial bifurcations and side branches prone to atherosclerosis. Enhanced oxidative stress and reduced endothelial NO production contribute significantly to the enhanced atherosclerosis at these regions.

the impact of Glu298Asp polymorphisms on atherosclerosis risk with partly promising results.[188,215–217] However, negative findings have also been reported.[184,218,219] Results from several meta-analyses indicate positive associations of Glu298Asp,[187–189] -786T>C,[189,190] and Intron 4[189] polymorphisms with CAD risk.

## Cardiovascular Risk Factors Induce Vascular Oxidative Stress and Decrease Endothelial NO Production

All established risk factors for atherosclerosis enhance oxidative stress and induces eNOS uncoupling in the vascular wall.[66,67] Uncoupling of eNOS leads to not only reduced endothelial NO production but also a potentiation of oxidative stress (Figure 2).

### Hypertension

Hypertension is a major risk factor for atherosclerosis and for CAD and stroke. One of the underlying mechanisms for the enhanced atherogenesis in hypertension patients is oxidative stress. In fact, oxidative stress plays a crucial role in the pathogenesis of hypertension itself. This has been shown for variety animal models of hypertension types, including angiotensin II–induced hypertension, spontaneously hypertensive rats (an animal model of genetic hypertension), and deoxycorticosterone acetate-salt hypertension, which is a low renin/angiotensin hypertension model. Moreover, NADPH oxidase is likely to represent the primary ROS source. Genetic deletion or pharmacological inhibition of NADPH oxidase lowers blood pressure in hypertension models.[8,220,221]

Uncoupled eNOS also contributes significantly to vascular oxidative stress in hypertension.[66,67] Molecular mechanisms for eNOS uncoupling in hypertension include BH4 deficiency, L-arginine deficiency, and S-glutathionylation. The deficiency of BH4 is caused by NADPH oxidase–mediated of BH4 oxidation[8] and by reduced BH4 recycling from BH2 because of a downregulation of endothelial dihydrofolate reductase.[222] L-Arginine deficiency in hypertension models has been attributed to upregulation of arginase expression/activity in blood vessels.[223–225] Uncoupling of eNOS by S-glutathionylation is evident in angiotensin II–induced hypertension.[226] Reversal of eNOS uncoupling reduces blood pressure in hypertensive

animals[221] or contributes to blood pressure reduction by some antihypertensive drugs.[226]

## Hypercholesterolemia

NADPH oxidases and XO are proposed to be the major sources of superoxide in the coronary artery of hypercholesterolemic patients with CAD.[55] Uncoupling of eNOS is likely to be a subsequent event secondary to oxidative stress mediated by NADPH oxidase (and XO) because of oxidation-induced BH4 deficiency. Both native LDL and oxLDL have been shown to stimulate superoxide/peroxynitrite production and to uncouple eNOS.[227,228] ROS production from uncoupled eNOS has been shown in LDL-treated endothelial cells, in hypercholesterolemic ApoE-KO mice[71] and in hypercholesterolemic patients.[72] In addition to the direct effects of LDL on endothelial ROS production, hypercholesterolemia may indirectly enhance oxidative stress by potentiating the effects of angiotensin II via upregulation of AT1 receptor.[229]

In addition to BH4 deficiency, L-arginine deficiency also represents a cause of eNOS uncoupling in hypercholesterolemia. An upregulation of arginase expression and activity has been shown in ApoE-KO mice (Arg2)[230,231] and in hyperlipidemic rabbits (Arg1 and Arg2).[232] The aortic arginase activity in ApoE-KO mice is significantly reduced after the removal of the endothelium, suggesting important contribution from endothelial cells.[230] The functional relevance of arginase upregulation in atherosclerosis has been shown in ApoE-KO mice. Selective endothelial overexpression of Arg2 induces endothelial dysfunction and enhances atherosclerosis in mice.[233] Chronic treatment with an arginase inhibitor for 4 or 8 weeks reduces aortic plaque burden in ApoE-KO mice.[230]

In addition to eNOS uncoupling, LDL and oxLDL also reduce endothelial NO production by inhibiting eNOS activity.[66] Hypercholesterolemia upregulates caveolin abundance, stimulates eNOS translocation to membrane caveolae, and promotes eNOS interaction with caveolin.[234] Moreover, LDL also decreases the association of eNOS with Hsp90.[228] These effects additively inhibit eNOS activity because caveolin is an inhibiting and Hsp90 a stimulating interaction protein of eNOS. Finally, oxLDL decreases eNOS activity by inhibiting Akt-mediated phosphorylation of eNOS at serine 1177[235] or by increased proteasomal degradation of eNOS protein.[236]

## Cigarette Smoking

Cigarette smoke–containing compounds have the potential to active endothelial NADPH oxidase[237] and to stimulate mitochondrial oxidative stress.[238] The enhanced production of superoxide and peroxynitrite leads to vascular inflammation, DNA damage, and vascular aging.[238] Moreover, compounds from cigarette smoke also induce oxidative modifications of LDL, which potentiates the pro-oxidative activity of LDL (oxLDL is more potent in activating NADPH oxidase than native LDL).[239] BH4 deficiency and eNOS uncoupling have been documented in smokers and supplementation with BH4 can reverse eNOS uncoupling and improve endothelial dysfunction in smokers.[75]

## Diabetes Mellitus

Hyperglycemia stimulates ROS production from different cellular sources. Among these, the mitochondrial electron-transport chain is likely to represent the initial superoxide producer.[240] Mitochondria-derived superoxide overproduction leads to activation of protein kinase C and formation of advanced glycation end products.[240] Protein kinase C and advanced glycation end products can activate NADPH oxidase and, at the same time, inhibit eNOS activity through post-translational modifications.[241]

Protein kinase C–stimulated NADPH oxidase activation and eNOS uncoupling have been observed in streptozotocin-induced type 1 diabetes mellitus.[242] This is likely to be attributable to NADPH oxidase–mediated BH4 oxidation. Indeed, BH4 oxidation and BH4 deficiency are evident in streptozotocin-treated mice[243] and rats.[244] In addition, diabetes mellitus also causes BH4 deficiency by reducing BH4 synthesis. Enhanced ROS production in diabetes mellitus accelerates proteasomal degradation of guanosine 5′-triphosphate cyclohydrolase I, a rate-limiting enzyme in the synthesis of BH4.[245–247] In addition, eNOS S-glutathionylation represents another important mechanism of eNOS uncoupling in the setting of type 1 diabetes mellitus.[247]

In mouse models of type 2 diabetes mellitus, a relative BH4 deficiency is evident because of an enhanced BH4 oxidation and a low BH4:BH2 ratio.[248–250] The increased levels of angiotensin II in diabetic patients may additionally reduce dihydrofolate reductase expression and decrease BH4 recycling from BH2.[222]

L-Arginine deficiency represents another mechanism for eNOS uncoupling under conditions of diabetes mellitus. High glucose upregulates Arg1 in (bovine and murine) endothelial cells,[251,252] whereas persistent insulin stimulation upregulates the expression and activity of Arg2 in human endothelial cells.[253] In addition, L-arginine deficiency and eNOS uncoupling have also been documented in rodent models of type 1[251,252,254,255] and type 2 diabetes mellitus.[256]

In patients with type 2 diabetes mellitus, plasma arginase activity is elevated.[257] An upregulation of Arg1 in coronary arterioles of patients with (type 1 or type 2) diabetes mellitus has been shown to contribute to the reduced NO production and consequently diminished vasodilation.[258] Arginase inhibition markedly improves endothelium-dependent vasodilation in the forearm of patients with type 2 diabetes mellitus and CAD, whereas it does not affect endothelial function in healthy controls.[259] This observation indicates a functional role of arginase contributing to endothelial dysfunction in patients with diabetes mellitus.

## Biomechanical Factors

Besides the risk factors at population level (hypertension, hypercholesterolemia, smoking, and diabetes mellitus), disturbed blood flow represents a key risk factor for atherosclerosis within an organism. Atherosclerosis preferentially develops at arches, arterial bifurcations, and side branches, regions that are exposed to nonuniform, disturbed patterns of blood flow.[260] Several studies have demonstrated the causal relationship between disturbed flow and atherosclerosis. Disturbed flow induced by applying a constrictive cuff[261,262]

or by partial ligation or tandem ligations[263,264] leads to rapid development of atherosclerosis in the carotid artery, which is otherwise resistant to atherogenesis.

In each cardiac cycle, arteries are exposed to perpendicular and longitudinal forces generated by intraluminal pressure, and axial stress (shear stress), which acts longitudinally on the surface of the arterial wall.[260] Whereas laminar flow (with a high shear stress parallel to the vascular wall and a low circumferential strain) is atheroprotective, disturbed flow (with a change in the diameter and proximity to bifurcations) is proatherogenic.[260,265,266] Endothelial cells exposed laminar flow show higher endothelia NO production and are resistant to inflammatory signals and have low intercellular permeability.[265] In contrast, endothelial cells exposed to disturbed flow show a proinflammatory phenotype with higher ROS production, elevated cell turnover, increased cell-cell permeability, and upregulated expression of adhesion molecules and chemokines.[265]

Laminar flow enhances endothelial NO production[267] by stimulating eNOS phosphorylation at serine 1177[268,269] and thus increasing the sensitivity of the enzyme to $Ca^{2+}$ so that the enzyme can be activated at resting $Ca^{2+}$ levels.[270] In addition, shear stress also upregulates eNOS expression.[271] In contrast, disturbed flow or oscillatory shear stress decreases eNOS expression and activity.[272,273]

On the contrary, oscillatory shear stress markedly increases endothelial superoxide production,[274] with NADPH oxidase being the primary superoxide source.[9,275] Importantly, NADPH oxidases serve a role as a master oxidase and promote ROS production from other enzymatic sources, eg, XO.[9] Xanthine oxidoreductase exists in 2 forms: xanthine dehydrogenase (XDH) and XO. XDH can be converted to XO by reversible sulfhydryl oxidation or by irreversible proteolytic modification.[276] In endothelial cells, oscillatory shear stress leads to a NADPH oxidase–dependent degradation of XDH resulting in an increase in XO/XDH ratio. Because both XO and XDH use xanthine as a substrate but only XO generates superoxide, the higher XO/XDH ratio results in enhanced XO-mediated superoxide production.[9]

## Redox Signaling

NO exerts its cellular effects through both cGMP-dependent and cGMP-independent mechanisms. In a cGMP-independent manner, NO modifies protein cysteine residues resulting in an S-nitrosothiol.[277] A plethora of proteins (including NADPH oxidase[278] and eNOS itself[279]) undergo S-nitrosylation, which may represent part of the mechanistical explanation of the wide range of cellular effects of NO in the cardiovascular system.[277]

Protein denitrosylation can be catalyzed by 2 major enzymatic systems. Whereas thioredoxins directly denitrosylate substrate S-nitrosothiol proteins, the S-nitrosoglutathione (GSNO) reductase governs protein S-nitrosylation indirectly by acting on GSNO and thereby modulates the cellular equilibrium between S-nitrosothiol proteins and GSNO.[277] Genetic deletion of GSNO reductase increases the levels of S-nitrosothiols in red blood cells and lowers systemic vascular resistance,[280] demonstrating the role for GSNO in conveying the systemic activity of NO derived from eNOS.[277] Interestingly, the endogenous gaseous signaling molecule hydrogen sulfide also regulates cysteine thiols,[281] protects against oxidative stress,[282] and has an impact on protein S-nitrosylation,[283,284] including S-nitrosylation of eNOS.[285]

Cysteine is a unique amino acid because of its thiol side chain, which is redox active. Numerous reactions are known to occur on cysteine thiol side chains that affect protein structure and function, including S-nitrosylation, S-sulfhydration (modification by hydrogen sulfide), S-glutathionylation, and disulfide bond formation. Moreover, stepwise oxidation of cysteine thiol by hydrogen peroxide results in the formation of sulfenic acid, sulfinic acid, and sulfonic acid.[286,287] Physiological conditions (NO>ROS) favor S-nitrosylation, whereas NO/ROS disequilibrium under oxidative stress favors oxidation reactions (S-glutathionylation, intramolecular disulfide, and sulfur oxides formation).[154] Thiol oxidation adversely impacts S-nitrosylation signaling.[154] Indeed, disruption of protein S-nitrosylation has been documented under conditions of heart failure[154,277] and high glucose.[288]

## Effects of ROS and NO in Key Steps of Atherosclerosis: The Molecular Links

ROS and NO are implicated in the initiation and propagation of atherosclerosis, which provides an explantation how cardiovascular risk factors promote atherogenesis.

### LDL Accumulation in the Vascular Wall

As mentioned above, atherosclerosis develops in the arterial system initially at predilection sites with geometries, such as arches, branches, and bifurcations.[289,290] One mechanism linking the disturbed flow patterns to atherogenesis is LDL accumulation in the vascular wall.

Theoretically, accumulation of LDL in the artery wall can be caused either by an increase in lipoprotein influx (increase in endothelial permeability and delivery of LDL into the artery wall) or a decrease in lipoprotein efflux caused by an increased binding LDL by the artery wall.

LDL transport from the circulation to the vessel wall is promoted at sites of disturbed flow because of flow stagnation and the subsequent prolonged contact between blood and vascular endothelial cells.[260,266] Moreover, structural examination of macromolecular structures reveals that a nearly confluent elastin surface layer is present throughout mouse aorta. This internal elastin layer, however, is missing at vascular branch points, which are among the sites most prone to atherosclerosis.[291] LDL binding is most extensive in the arterial branch points where the elastin layer is absent,[291] indicating that the absence of an elastin layer contributes to the initial LDL infiltration at these sites and the subsequent development of atherosclerosis.

A recent study has provided compelling evidence that LDL retention represents a mechanism that could be even more important than LDL influx for LDL accumulation in flow-disturbed vascular regions.[289] Infusion of normocholesterolemic mice with labeled human LDL results in LDL retention in the intimal and medial layers along the inner curvature of the aortic arch and at branch points.[289] The straight segment of the common carotid artery is exposed to uniform laminar flow and is resistant to atherosclerosis. Normally, no LDL retention is found in this region.[289] Implanting a mildly

constrictive perivascular collar evokes disturbed laminar flow in the segment proximal to the collar characterized by low wall shear stress and cyclic circumferential stretching.[292] Strikingly, this manipulated blood flow in the straight segment of the common carotid artery is sufficient to transform this otherwise atherosclerosis-resistant site into LDL-retaining regions. Importantly, the accumulation of LDL in the flow-manipulated region is a result of enhanced LDL binding by the vascular wall without any changes in endothelial permeability or LDL influx.[289] Gene expression analyses have revealed that flow manipulation leads to increased expression of proteoglycan core proteins associated with LDL binding and retention.[289]

The expression of proteoglycans is upregulated after vascular injury[293] and in insulin resistance[294] associated with increased cholesterol deposition in the vascular wall. Proatherogenic molecules such as platelet-derived growth factor stimulates proteoglycan synthesis in VSMC and enhances LDL retention in blood vessels.[295,296] Interestingly, proteoglycans secreted from statin-exposed cells demonstrate a reduction in LDL-binding affinity. Thus, the atheroprotective effects of statins may be partly attributed to changes in vascular proteoglycans and lower LDL retention in the vessel wall.[297]

The roles of NO and oxidative stress in LDL accumulation in the vascular wall have not been sufficiently studied to date. Previous studies have shown that endothelial NO prevents the uptake of LDL by arterial walls.[298,299] Disturbed flow is known to reduce endothelial NO production[300] and enhance ROS production in endothelial cells and in VSMC.[9,274,301,302] However, the precise roles of NO and ROS in LDL uptake and LDL binding (eg, regulation of proteoglycan expression) are still unknown and warrants future studies.

## LDL Oxidation

Experimental studies have demonstrated that oxLDL within the arterial wall promotes atherogenesis,[303–305] although direct evidence for the role of LDL oxidation in human atherosclerosis is still rare[306] and many questions remain to be answered.[305]

OxLDL exhibits a wide array of proatherogenic properties.[304] Many of these effects are mediated by oxidized phospholipids within the LDL molecules.[304] Lipid peroxidation can occur through nonenzymatic mechanisms (eg, by ROS derived from NADPH oxidase or uncoupled eNOS)[307] or through enzymatic mechanisms (eg, by myeloperoxidases, lipoxygenases, cyclooxygenases, and cytochrome P450).[308] The lipid peroxidation products, such as malondialdehyde, 4-hydroxynonenal, phosphocholine of oxidized phospholipid, and 2-(ω-carboxyethyl) pyrrole, are highly reactive. They modify self-molecules leading to the generation of structural neoepitopes termed oxidation-specific epitopes (OSEs).[308] OSEs, including oxidized phospholipids and malondialdehyde-modified amino groups, have been documented on the surface of apoptotic cells and oxLDL molecules.[303,308]

Peroxidation of phospholipids promotes a conformational change in the apoB-100 molecule leading to increased nonreceptor-mediated capture of the oxLDL particle by vascular cells.[303] Moreover, OSEs are recognized by both cellular (eg, scavenger receptors and toll-like receptors [TLRs]) and soluble pattern recognition receptors (eg, proteins of the complement system, C-reactive protein, and natural IgM antibodies).[308]

The recognition of OSEs by cellular and humoral immune responses has important physiological roles in maintaining tissue homeostasis by removing dying cells, cellular debris, and damaged molecules.[308] In situations of increased oxidative stress, however, OSEs generation is significantly increased. Furnished with a variety of scavenger receptors and TLRs, endothelial cells, and macrophages are the major cellular sensors of OSEs in atherosclerosis. Consequently, the persistent sensing of OSEs by these cells triggers chronic inflammation through the secretion of chemokines and proinflammatory cytokines (see below).

The role of LDL oxidation in atherogenesis has been shown in gene targeting studies.[304] Genetic deletion of lipoxygenases decreases LDL oxidation and atherosclerosis lesion in mouse models of atherosclerosis.[309–312] Disruption of paraoxonase 1, an enzyme that indirectly inhibits LDL oxidation, increases LDL oxidation and lesion formation.[313] Moreover, OSE-specific natural IgM antibodies block the binding and uptake of oxLDL by macrophages, prevent foam cell formation,[314] and decrease atherosclerosis in mice.[315,316] Patients with lower levels of IgM antibodies directed against malondialdehyde-LDL and oxLDL show an increased risk of cardiovascular disease.[317]

Interestingly, lipid peroxidation also leads to the formation of highly reactive γ-ketoaldehydes (isoketals). Proteins oxidatively modified by isoketals are formed in hypertension and accumulate in dendritic cells. Peptides derived from isoketal adducts of proteins behave as modified self-antigens, activating dendritic cells and T cells, which has been recently identified as a novel mechanism in hypertension.[318,319]

As aforementioned, NADPH oxidase–derived ROS are involved in LDL oxidation.[275] Interestingly, results from bone marrow transplantation experiments in mice indicate that NADPH oxidase in infiltrating immune cells in the vascular wall may be of more importance in LDL oxidation than those in the resident cells of the vascular wall.[49] In contrast, endothelial NO has been shown to inhibit LDL oxidation[320,321]

## Endothelial Cell Activation and Adhesion Molecule Expression

Endothelial cells can sense OSE and take up oxLDL via the lectin-like oxidized LDL receptor-1, TLR2, and TLR4.[303,308] This may lead to a variety of biological effects, such as (1) reduction of endothelial NO production, (2) upregulation of leukocyte adhesion molecules, (3) promotion of a prothrombotic surface, and (4) synthesis of smooth muscle cell mitogenic factors.[303]

OSE sensing by endothelial cells is a key response in the development of atherosclerosis.[308] Oxidized phospholipids, for instance, induce the expression of chemoattractants (eg, MCP-1 [monocyte chemoattractant protein-1], chemokine (C-X-C motif) ligand 8 and P selectin) and trigger monocyte binding to endothelial cells via TLR4.[307] 4-hydroxynonenal induces nuclear factor-κB activation in endothelial cells which is mediated by LOX1.[322] 2-(ω-carboxyethyl) pyrrole has been

shown to activate endothelial cells by stimulating TLR2.[323] Deficiency of LOX1[324,325] or TLR2[326] reduces vascular cell adhesion molecule-1 expression and atherosclerosis in LDL receptor-KO mice.

The expression of adhesion molecules by endothelial cells is inhibited by laminar shear stress,[327] but enhanced by disturbed flow.[328,329] In addition, endothelial expression of adhesion molecules is also upregulated by proatherogenic stimuli, such as proinflammatory cytokines,[330,331] angiotensin II,[332] advanced glycation end products,[333] and leptin.[334] Interestingly, the anti-inflammatory adipokine, adiponectin, inhibits the expression of adhesion molecules in endothelial cells and prevents leukocyte–endothelium interaction.[335]

NO and ROS have opposite roles in the regulation of adhesion molecule expression and endothelial–leukocyte interaction. Endothelial NO inhibits cytokine-induced nuclear factor-κB activation and upregulation of vascular cell adhesion molecule-1, E-selectin, and intercellular adhesion molecule-1.[132,331,336–338] Inhibition of NO production increases leukocyte adherence, indicating that endothelial NO is an endogenous modulator of leukocyte adhesion to vascular endothelium.[339] On the contrary, ROS are implicated in upregulation of adhesion molecules induced by cytokines[330,331] or by leptin.[334] ROS not only potentiate OSE sensing by upregulation of endothelial TLR2 expression,[326] but are also involved in the intercellular signaling cascades leading to adhesion molecules expression induced by stretch or oscillatory shear stress.[340,341]

In agreement with this concept, targeted delivery of SOD to endothelial cells in mice inhibits nuclear factor-κB signaling and vascular cell adhesion molecule-1 expression.[342] In contrast, genetic disruption of peroxiredoxin1[343] or GPx1[344] potentiates chemoattractant expression and leukocyte–endothelial cell adhesion.

### Macrophage Activation and Foam Cell Formation

A hallmark of atherosclerosis is the formation of foam cells due to enhanced uptake of oxLDL by macrophages mediated by OSE binding to scavenger receptors (eg, CD36, TLRs, SRA1 [scavenger receptor A1] and lectin-like oxidized LDL receptor-1).[308,345] OxLDL is taken up much more rapidly than native LDL by monocytes/macrophages.[304] The scavenger receptors are not downregulated by intracellular LDL, allowing progressive accumulation of cholesterol to the point of foam cell generation.[304]

The uptake of oxLDL by macrophages has an important physiological role, as this facilitates oxLDL removal.[308] However, under pathological situations, the enhanced uptake of cholesterol-rich LDL and subsequent inefficient removal of intracellular cholesterol can trigger signaling pathways that induce the secretion of inflammatory chemokines and cytokines.[308] SRA and CD36 account for ≈90% of macrophage uptake of oxLDL. Genetic disruption of either CD36 or SRA1 reduces atherosclerosis in mice.[304,345,346] Supersaturation of cholesterol in macrophages results in the formation of cholesterol crystals leading to the damage of lysosomes, inflammasome activation, and interleukin-1β secretion.[347] Excessive foam cell formation induces macrophage apoptosis as observed in advanced atherosclerotic lesions.[308,348]

Oxidative stress plays a crucial role in macrophage activation and foam cell formation. ROS derived from NADPH oxidase and uncoupled eNOS are involved in the generation of OSE.[307] Moreover, XO also plays an important role in cholesterol crystal-induced ROS formation and inflammatory cytokine release by macrophages. XO inhibition reduces arterial ROS levels, improves endothelial dysfunction, and suppresses plaque formation in ApoE-KO mice.[51]

## Therapeutic Strategies

Despite the role of oxidative stress in the pathogenesis of atherosclerosis and other diseases, dietary antioxidant supplements to human subjects do show preventative or therapeutic effect. In a large meta-analysis, antioxidant supplements show no benefit (vitamin C and selenium) or even increase mortality (beta carotene, vitamin A, and vitamin E).[349,350]

However, the ineffectiveness of the antioxidant therapy does not disprove the role of oxidative stress in atherogenesis. Rather, the used antioxidant compounds are likely to be unspecific, not correctly dosed, cannot reach the intracellular compartment of ROS generation, and some may even have pro-oxidative properties.[4,351,352] Therefore, better antioxidant strategies need to be developed.

Among the ROS-producing enzymes, NADPH oxidases are proposed to be the master oxidases.[9] NADPH oxidase activation promotes ROS production from other sources. Therefore, NADPH oxidases represent attractive therapeutic targets. However, because of the protective role of Nox4 shown in recent studies, a promising Nox inhibitor should be specific for Nox1 or Nox2. Nox inhibitors with improved specificity for NADPH oxidases and moderate Nox isoform selectivity have been developed.[353] The first results in mouse atherosclerosis models are encouraging.

In addition to inhibition of XO activity[51] and prevention of mitochondrial oxidative stress,[354] pharmacological reversal of eNOS uncoupling represents further fascinating strategies. Among the drugs currently in clinical use, inhibitors of the renin–angiotensin–aldosterone system, statins, nebivolol, and pentaerythritol tetranitrate have been shown to prevent or reverse eNOS uncoupling under experimental conditions. Other compounds, such as resveratrol, sepiapterin, folic acid, and AVE3085, may also recouple eNOS and improve endothelial function although the long-term benefit of these compounds is still unknown (see our recent reviews[66,67,355]).

## Conclusion

Endothelial NO protects against, whereas vascular oxidative stress promotes, atherosclerosis. Cardiovascular risk factors decrease endothelial NO production and stimulate ROS production from various ROS sources including NADPH oxidases, XO, mitochondria, and uncoupled eNOS. ROS and NO have opposite roles in the process of atherogenesis, such as LDL oxidation, endothelial cell activation, and macrophage infiltration/activation. Prevention of vascular oxidative stress and improvement of endothelia NO production may represent feasible therapeutic strategies in addition to the treatment of established cardiovascular risk factors.

## Acknowledgments

Original work from the authors' laboratory contributing to this review was supported by the German Research Foundation (DFG; LI-1042/1–1 and LI-1042/3-1), the German Federal Ministry of Education and Research (BMBF; 01EO1003), and intramural fund (Stufe I) of the Johannes Gutenberg University Medical Center Mainz.

## Disclosures

None.

## References

1. Stocker R, Keaney JF Jr. Role of oxidative modifications in atherosclerosis. *Physiol Rev*. 2004;84:1381–1478. doi: 10.1152/physrev.00047.2003.
2. Förstermann U. Oxidative stress in vascular disease: causes, defense mechanisms and potential therapies. *Nat Clin Pract Cardiovasc Med*. 2008;5:338–349. doi: 10.1038/ncpcardio1211.
3. Förstermann U. Nitric oxide and oxidative stress in vascular disease. *Pflugers Arch*. 2010;459:923–939. doi: 10.1007/s00424-010-0808-2.
4. Li H, Horke S, Förstermann U. Vascular oxidative stress, nitric oxide and atherosclerosis. *Atherosclerosis*. 2014;237:208–219. doi: 10.1016/j.atherosclerosis.2014.09.001.
5. Daiber A, Di Lisa F, Oelze M, Kroller-Schon S, Steven S, Schulz E, Munzel T. Crosstalk of mitochondria with nadph oxidase via reactive oxygen and nitrogen species signalling and its role for vascular function [published online ahead of print December 9, 2015]. *Br J Pharmacol*. doi: 10.1111/bph.13403. http://onlinelibrary.wiley.com/doi/10.1111/bph.13403/abstract;jsessionid=150E9A30F843D95F53F162452858D588.f04t04.
6. Daiber A. Redox signaling (cross-talk) from and to mitochondria involves mitochondrial pores and reactive oxygen species. *Biochim Biophys Acta*. 2010;1797:897–906. doi: 10.1016/j.bbabio.2010.01.032.
7. Itani HA, Dikalova AE, McMaster WG, Nazarewicz RR, Bikineyeva AT, Harrison DG, Dikalov SI. Mitochondrial cyclophilin D in vascular oxidative stress and hypertension. *Hypertension*. 2016;67:1218–1227. doi: 10.1161/HYPERTENSIONAHA.115.07085.
8. Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, Mitch WE, Harrison DG. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J Clin Invest*. 2003;111:1201–1209. doi: 10.1172/JCI14172.
9. McNally JS, Davis ME, Giddens DP, Saha A, Hwang J, Dikalov S, Jo H, Harrison DG. Role of xanthine oxidoreductase and NAD(P)H oxidase in endothelial superoxide production in response to oscillatory shear stress. *Am J Physiol Heart Circ Physiol*. 2003;285:H2290–H2297. doi: 10.1152/ajpheart.00515.2003.
10. Landmesser U, Spiekermann S, Preuss C, Sorrentino S, Fischer D, Manes C, Mueller M, Drexler H. Angiotensin II induces endothelial xanthine oxidase activation: role for endothelial dysfunction in patients with coronary disease. *Arterioscler Thromb Vasc Biol*. 2007;27:943–948. doi: 10.1161/01.ATV.0000258415.32883.bf.
11. Dikalova AE, Bikineyeva AT, Budzyn K, Nazarewicz RR, McCann L, Lewis W, Harrison DG, Dikalov SI. Therapeutic targeting of mitochondrial superoxide in hypertension. *Circ Res*. 2010;107:106–116. doi: 10.1161/CIRCRESAHA.109.214601.
12. Kröller-Schön S, Steven S, Kossmann S, et al. Molecular mechanisms of the crosstalk between mitochondria and NADPH oxidase through reactive oxygen species-studies in white blood cells and in animal models. *Antioxid Redox Signal*. 2014;20:247–266. doi: 10.1089/ars.2012.4953.
13. Wenzel P, Mollnau H, Oelze M, Schulz E, Wickramanayake JM, Müller J, Schuhmacher S, Hortmann M, Baldus S, Gori T, Brandes RP, Münzel T, Daiber A. First evidence for a crosstalk between mitochondrial and NADPH oxidase-derived reactive oxygen species in nitroglycerin-triggered vascular dysfunction. *Antioxid Redox Signal*. 2008;10:1435–1447. doi: 10.1089/ars.2007.1969.
14. Doughan AK, Harrison DG, Dikalov SI. Molecular mechanisms of angiotensin II-mediated mitochondrial dysfunction: linking mitochondrial oxidative damage and vascular endothelial dysfunction. *Circ Res*. 2008;102:488–496. doi: 10.1161/CIRCRESAHA.107.162800.
15. Drummond GR, Selemidis S, Griendling KK, Sobey CG. Combating oxidative stress in vascular disease: NADPH oxidases as therapeutic targets. *Nat Rev Drug Discov*. 2011;10:453–471. doi: 10.1038/nrd3403.
16. Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev*. 2007;87:245–313. doi: 10.1152/physrev.00044.2005.
17. Brandes RP, Kreuzer J. Vascular NADPH oxidases: molecular mechanisms of activation. *Cardiovasc Res*. 2005;65:16–27. doi: 10.1016/j.cardiores.2004.08.007.
18. Schröder K, Zhang M, Benkhoff S, Mieth A, Pliquett R, Kosowski J, Kruse C, Luedike P, Michaelis UR, Weissmann N, Dimmeler S, Shah AM, Brandes RP. Nox4 is a protective reactive oxygen species generating vascular NADPH oxidase. *Circ Res*. 2012;110:1217–1225. doi: 10.1161/CIRCRESAHA.112.267054.
19. Lassègue B, Sorescu D, Szöcs K, Yin Q, Akers M, Zhang Y, Grant SL, Lambeth JD, Griendling KK. Novel gp91(phox) homologues in vascular smooth muscle cells: nox1 mediates angiotensin II-induced superoxide formation and redox-sensitive signaling pathways. *Circ Res*. 2001;88:888–894.
20. Ellmark SH, Dusting GJ, Fui MN, Guzzo-Pernell N, Drummond GR. The contribution of Nox4 to NADPH oxidase activity in mouse vascular smooth muscle. *Cardiovasc Res*. 2005;65:495–504. doi: 10.1016/j.cardiores.2004.10.026.
21. Görlach A, Brandes RP, Nguyen K, Amidi M, Dehghani F, Busse R. A gp91phox containing NADPH oxidase selectively expressed in endothelial cells is a major source of oxygen radical generation in the arterial wall. *Circ Res*. 2000;87:26–32.
22. Ago T, Kitazono T, Ooboshi H, Iyama T, Han YH, Takada J, Wakisaka M, Ibayashi S, Utsumi H, Iida M. Nox4 as the major catalytic component of an endothelial NAD(P)H oxidase. *Circulation*. 2004;109:227–233. doi: 10.1161/01.CIR.0000105680.92873.70.
23. Xu H, Goettsch C, Xia N, Horke S, Morawietz H, Förstermann U, Li H. Differential roles of PKCalpha and PKCepsilon in controlling the gene expression of Nox4 in human endothelial cells. *Free Radic Biol Med*. 2008;44:1656–1667. doi: 10.1016/j.freeradbiomed.2008.01.023.
24. Fulton DJ, Barman SA. Clarity on the isoform-specific roles of NADPH oxidases and NADPH oxidase-4 in atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2016;36:579–581. doi: 10.1161/ATVBAHA.116.307096.
25. Sheehan AL, Carrell S, Johnson B, Stanic B, Banfi B, Miller FJ Jr. Role for Nox1 NADPH oxidase in atherosclerosis. *Atherosclerosis*. 2011;216:321–326. doi: 10.1016/j.atherosclerosis.2011.02.028.
26. Gray SP, Di Marco E, Okabe J, et al. NADPH oxidase 1 plays a key role in diabetes mellitus-accelerated atherosclerosis. *Circulation*. 2013;127:1888–1902. doi: 10.1161/CIRCULATIONAHA.112.132159.
27. Sobey CG, Judkins CP, Rivera J, Lewis CV, Diep H, Lee HW, Kemp-Harper BK, Broughton BR, Selemidis S, Gaspari TA, Samuel CS, Drummond GR. NOX1 deficiency in apolipoprotein E-knockout mice is associated with elevated plasma lipids and enhanced atherosclerosis. *Free Radic Res*. 2015;49:186–198. doi: 10.3109/10715762.2014.992893.
28. Gray SP, Di Marco E, Kennedy K, Chew P, Okabe J, El-Osta A, Calkin AC, Biessen EA, Touyz RM, Cooper ME, Schmidt HH, Jandeleit-Dahm KA. Reactive oxygen species can provide atheroprotection via NOX4-dependent inhibition of inflammation and vascular remodeling. *Arterioscler Thromb Vasc Biol*. 2016;36:295–307. doi: 10.1161/ATVBAHA.115.307012.
29. Quesada IM, Lucero A, Amaya C, Meijles DN, Cifuentes ME, Pagano PJ, Castro C. Selective inactivation of NADPH oxidase 2 causes regression of vascularization and the size and stability of atherosclerotic plaques. *Atherosclerosis*. 2015;242:469–475. doi: 10.1016/j.atherosclerosis.2015.08.011.
30. Kirk EA, Dinauer MC, Rosen H, Chait A, Heinecke JW, LeBoeuf RC. Impaired superoxide production due to a deficiency in phagocyte NADPH oxidase fails to inhibit atherosclerosis in mice. *Arterioscler Thromb Vasc Biol*. 2000;20:1529–1535.
31. Judkins CP, Diep H, Broughton BR, Mast AE, Hooker EU, Miller AA, Selemidis S, Dusting GJ, Sobey CG, Drummond GR. Direct evidence of a role for Nox2 in superoxide production, reduced nitric oxide bioavailability, and early atherosclerotic plaque formation in ApoE-/- mice. *Am J Physiol Heart Circ Physiol*. 2010;298:H24–H32. doi: 10.1152/ajpheart.00799.2009.
32. Hsich E, Segal BH, Pagano PJ, Rey FE, Paigen B, Deleonardis J, Hoyt RF, Holland SM, Finkel T. Vascular effects following homozygous disruption of p47(phox): An essential component of NADPH oxidase. *Circulation*. 2000;101:1234–1236.
33. Barry-Lane PA, Patterson C, van der Merwe M, Hu Z, Holland SM, Yeh ET, Runge MS. p47phox is required for atherosclerotic lesion progression in ApoE(-/-) mice. *J Clin Invest*. 2001;108:1513–1522. doi: 10.1172/JCI11927.
34. Douglas G, Bendall JK, Crabtree MJ, Tatham AL, Carter EE, Hale AB, Channon KM. Endothelial-specific Nox2 overexpression increases vascular superoxide and macrophage recruitment in ApoE$^{-/-}$ mice. *Cardiovasc Res*. 2012;94:20–29. doi: 10.1093/cvr/cvs026.

35. Craige SM, Kant S, Reif M, Chen K, Pei Y, Angoff R, Sugamura K, Fitzgibbons T, Keaney JF Jr. Endothelial NADPH oxidase 4 protects ApoE-/- mice from atherosclerotic lesions. *Free Radic Biol Med*. 2015;89:1–7. doi: 10.1016/j.freeradbiomed.2015.07.004.
36. Schürmann C, Rezende F, Kruse C, Yasar Y, Löwe O, Fork C, van de Sluis B, Bremer R, Weissmann N, Shah AM, Jo H, Brandes RP, Schröder K. The NADPH oxidase Nox4 has anti-atherosclerotic functions. *Eur Heart J*. 2015;36:3447–3456. doi: 10.1093/eurheartj/ehv460.
37. Langbein H, Brunssen C, Hofmann A, Cimalla P, Brux M, Bornstein SR, Deussen A, Koch E, Morawietz H. NADPH oxidase 4 protects against development of endothelial dysfunction and atherosclerosis in LDL receptor deficient mice. *Eur Heart J*. 2016;37:1753–1761. doi: 10.1093/eurheartj/ehv564.
38. Takac I, Schröder K, Zhang L, Lardy B, Anilkumar N, Lambeth JD, Shah AM, Morel F, Brandes RP. The E-loop is involved in hydrogen peroxide formation by the NADPH oxidase Nox4. *J Biol Chem*. 2011;286:13304–13313. doi: 10.1074/jbc.M110.192138.
39. Craige SM, Chen K, Pei Y, Li C, Huang X, Chen C, Shibata R, Sato K, Walsh K, Keaney JF Jr. NADPH oxidase 4 promotes endothelial angiogenesis through endothelial nitric oxide synthase activation. *Circulation*. 2011;124:731–740. doi: 10.1161/CIRCULATIONAHA.111.030775.
40. Di Marco E, Gray SP, Kennedy K, Szyndralewiez C, Lyle AN, Lassègue B, Griendling KK, Cooper ME, Schmidt HH, Jandeleit-Dahm KA. NOX4-derived reactive oxygen species limit fibrosis and inhibit proliferation of vascular smooth muscle cells in diabetic atherosclerosis. *Free Radic Biol Med*. 2016;97:556–567. doi: 10.1016/j.freeradbiomed.2016.07.013.
41. Kleinschnitz C, Grund H, Wingler K, Armitage ME, Jones E, Mittal M, Barit D, Schwarz T, Geis C, Kraft P, Barthel K, Schuhmann MK, Herrmann AM, Meuth SG, Stoll G, Meurer S, Schrewe A, Becker L, Gailus-Durner V, Fuchs H, Klopstock T, de Angelis MH, Jandeleit-Dahm K, Shah AM, Weissmann N, Schmidt HH. Post-stroke inhibition of induced nadph oxidase type 4 prevents oxidative stress and neurodegeneration. *PLoS Biol*. 2010;8
42. Nishimura A, Ago T, Kuroda J, Arimura K, Tachibana M, Nakamura K, Wakisaka Y, Sadoshima J, Iihara K, Kitazono T. Detrimental role of pericyte Nox4 in the acute phase of brain ischemia. *J Cereb Blood Flow Metab*. 2016;36:1143–1154. doi: 10.1177/0271678X15606456.
43. Kuroda J, Ago T, Matsushima S, Zhai P, Schneider MD, Sadoshima J. NADPH oxidase 4 (Nox4) is a major source of oxidative stress in the failing heart. *Proc Natl Acad Sci U S A*. 2010;107:15565–15570. doi: 10.1073/pnas.1002178107.
44. Maalouf RM, Eid AA, Gorin YC, Block K, Escobar GP, Bailey S, Abboud HE. Nox4-derived reactive oxygen species mediate cardiomyocyte injury in early type 1 diabetes. *Am J Physiol Cell Physiol*. 2012;302:C597–C604. doi: 10.1152/ajpcell.00331.2011.
45. Guzik TJ, Chen W, Gongora MC, Guzik B, Lob HE, Mangalat D, Hoch N, Dikalov S, Rudzinski P, Kapelak B, Sadowski J, Harrison DG. Calcium-dependent NOX5 nicotinamide adenine dinucleotide phosphate oxidase contributes to vascular oxidative stress in human coronary artery disease. *J Am Coll Cardiol*. 2008;52:1803–1809. doi: 10.1016/j.jacc.2008.07.063.
46. Yu P, Han W, Villar VA, Yang Y, Lu Q, Lee H, Li F, Quinn MT, Gildea JJ, Felder RA, Jose PA. Unique role of NADPH oxidase 5 in oxidative stress in human renal proximal tubule cells. *Redox Biol*. 2014;2:570–579. doi: 10.1016/j.redox.2014.01.020.
47. Holterman CE, Thibodeau JF, Towaij C, Gutsol A, Montezano AC, Parks RJ, Cooper ME, Touyz RM, Kennedy CR. Nephropathy and elevated BP in mice with podocyte-specific NADPH oxidase 5 expression. *J Am Soc Nephrol*. 2014;25:784–797. doi: 10.1681/ASN.2013040371.
48. Montezano AC, Tsiropoulou S, Dulak-Lis M, Harvey A, Camargo Lde L, Touyz RM. Redox signaling, Nox5 and vascular remodeling in hypertension. *Curr Opin Nephrol Hypertens*. 2015;24:425–433. doi: 10.1097/MNH.0000000000000153.
49. Vendrov AE, Hakim ZS, Madamanchi NR, Rojas M, Madamanchi C, Runge MS. Atherosclerosis is attenuated by limiting superoxide generation in both macrophages and vessel wall cells. *Arterioscler Thromb Vasc Biol*. 2007;27:2714–2721. doi: 10.1161/ATVBAHA.107.152629.
50. Nishino T, Okamoto K, Eger BT, Pai EF, Nishino T. Mammalian xanthine oxidoreductase - mechanism of transition from xanthine dehydrogenase to xanthine oxidase. *FEBS J*. 2008;275:3278–3289. doi: 10.1111/j.1742-4658.2008.06489.x.
51. Nomura J, Busso N, Ives A, Matsui C, Tsujimoto S, Shirakura T, Tamura M, Kobayashi T, So A, Yamanaka Y. Xanthine oxidase inhibition by febuxostat attenuates experimental atherosclerosis in mice. *Sci Rep*. 2014;4:4554. doi: 10.1038/srep04554.
52. White CR, Darley-Usmar V, Berrington WR, McAdams M, Gore JZ, Thompson JA, Parks DA, Tarpey MM, Freeman BA. Circulating plasma xanthine oxidase contributes to vascular dysfunction in hypercholesterolemic rabbits. *Proc Natl Acad Sci U S A*. 1996;93:8745–8749.
53. Ohara Y, Peterson TE, Harrison DG. Hypercholesterolemia increases endothelial superoxide anion production. *J Clin Invest*. 1993;91:2546–2551. doi: 10.1172/JCI116491.
54. Patetsios P, Song M, Shutze WP, Pappas C, Rodino W, Ramirez JA, Panetta TF. Identification of uric acid and xanthine oxidase in atherosclerotic plaque. *Am J Cardiol*. 2001;88:188–91, A6.
55. Guzik TJ, Sadowski J, Guzik B, Jopek A, Kapelak B, Przybylowski P, Wierzbicki K, Korbut R, Harrison DG, Channon KM. Coronary artery superoxide production and nox isoform expression in human coronary artery disease. *Arterioscler Thromb Vasc Biol*. 2006;26:333–339. doi: 10.1161/01.ATV.0000196651.64776.51.
56. Guthikonda S, Sinkey C, Barenz T, Haynes WG. Xanthine oxidase inhibition reverses endothelial dysfunction in heavy smokers. *Circulation*. 2003;107:416–421.
57. Schröder K, Vecchione C, Jung O, Schreiber JG, Shiri-Sverdlov R, van Gorp PJ, Busse R, Brandes RP. Xanthine oxidase inhibitor tungsten prevents the development of atherosclerosis in ApoE knockout mice fed a Western-type diet. *Free Radic Biol Med*. 2006;41:1353–1360. doi: 10.1016/j.freeradbiomed.2006.03.026.
58. Wang Y, Wang GZ, Rabinovitch PS, Tabas I. Macrophage mitochondrial oxidative stress promotes atherosclerosis and nuclear factor-κB-mediated inflammation in macrophages. *Circ Res*. 2014;114:421–433. doi: 10.1161/CIRCRESAHA.114.302153.
59. Corral-Debrinski M, Shoffner JM, Lott MT, Wallace DC. Association of mitochondrial DNA damage with aging and coronary atherosclerotic heart disease. *Mutat Res*. 1992;275:169–180.
60. Li Y, Huang TT, Carlson EJ, Melov S, Ursell PC, Olson JL, Noble LJ, Yoshimura MP, Berger C, Chan PH, Wallace DC, Epstein CJ. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat Genet*. 1995;11:376–381. doi: 10.1038/ng1295-376.
61. Nojiri H, Shimizu T, Funakoshi M, Yamaguchi O, Zhou H, Kawakami S, Ohta Y, Sami M, Tachibana T, Ishikawa H, Kurosawa H, Kahn RC, Otsu K, Shirasawa T. Oxidative stress causes heart failure with impaired mitochondrial respiration. *J Biol Chem*. 2006;281:33789–33801. doi: 10.1074/jbc.M602118200.
62. Ballinger SW, Patterson C, Knight-Lozano CA, Burow DL, Conklin CA, Hu Z, Reuf J, Horaist C, Lebovitz R, Hunter GC, McIntyre K, Runge MS. Mitochondrial integrity and function in atherogenesis. *Circulation*. 2002;106:544–549.
63. Li H, Förstermann U. Nitric oxide in the pathogenesis of vascular disease. *J Pathol*. 2000;190:244–254. doi: 10.1002/(SICI)1096-9896(200002)190:3<244::AID-PATH575>3.0.CO;2-8.
64. Li H, Förstermann U. Prevention of atherosclerosis by interference with the vascular nitric oxide system. *Curr Pharm Des*. 2009;15:3133–3145.
65. Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function. *Eur Heart J*. 2012;33:829–37, 837a. doi: 10.1093/eurheartj/ehr304.
66. Li H, Förstermann U. Uncoupling of endothelial NO synthase in atherosclerosis and vascular disease. *Curr Opin Pharmacol*. 2013;13:161–167. doi: 10.1016/j.coph.2013.01.006.
67. Li H, Horke S, Förstermann U. Oxidative stress in vascular disease and its pharmacological prevention. *Trends Pharmacol Sci*. 2013;34:313–319. doi: 10.1016/j.tips.2013.03.007.
68. Förstermann U, Münzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. *Circulation*. 2006;113:1708–1714. doi: 10.1161/CIRCULATIONAHA.105.602532.
69. Alp NJ, McAteer MA, Khoo J, Choudhury RP, Channon KM. Increased endothelial tetrahydrobiopterin synthesis by targeted transgenic GTP-cyclohydrolase I overexpression reduces endothelial dysfunction and atherosclerosis in ApoE-knockout mice. *Arterioscler Thromb Vasc Biol*. 2004;24:445–450. doi: 10.1161/01.ATV.0000115637.48689.77.
70. Wohlfart P, Xu H, Endlich A, Habermeier A, Closs EI, Hübschle T, Mang C, Strobel H, Suzuki T, Kleinert H, Förstermann U, Ruetten H, Li H. Antiatherosclerotic effects of small-molecular-weight compounds enhancing endothelial nitric-oxide synthase (eNOS) expression and preventing eNOS uncoupling. *J Pharmacol Exp Ther*. 2008;325:370–379. doi: 10.1124/jpet.107.128009.
71. Xia N, Daiber A, Habermeier A, Closs EI, Thum T, Spanier G, Lu Q, Oelze M, Torzewski M, Lackner KJ, Münzel T, Förstermann U, Li H. Resveratrol reverses endothelial nitric-oxide synthase uncoupling in apolipoprotein E knockout mice. *J Pharmacol Exp Ther*. 2010;335:149–154. doi: 10.1124/jpet.110.168724.

72. Stroes E, Kastelein J, Cosentino F, Erkelens W, Wever R, Koomans H, Lüscher T, Rabelink T. Tetrahydrobiopterin restores endothelial function in hypercholesterolemia. *J Clin Invest*. 1997;99:41–46. doi: 10.1172/JCI119131.
73. Antoniades C, Shirodaria C, Crabtree M, Rinze R, Alp N, Cunnington C, Diesch J, Tousoulis D, Stefanadis C, Leeson P, Ratnatunga C, Pillai R, Channon KM. Altered plasma versus vascular biopterins in human atherosclerosis reveal relationships between endothelial nitric oxide synthase coupling, endothelial function, and inflammation. *Circulation*. 2007;116:2851–2859. doi: 10.1161/CIRCULATIONAHA.107.704155.
74. Porkert M, Sher S, Reddy U, Cheema F, Niessner C, Kolm P, Jones DP, Hooper C, Taylor WR, Harrison D, Quyyumi AA. Tetrahydrobiopterin: a novel antihypertensive therapy. *J Hum Hypertens*. 2008;22:401–407. doi: 10.1038/sj.jhh.1002329.
75. Heitzer T, Krohn K, Albers S, Meinertz T. Tetrahydrobiopterin improves endothelium-dependent vasodilation by increasing nitric oxide activity in patients with Type II diabetes mellitus. *Diabetologia*. 2000;43:1435–1438. doi: 10.1007/s001250051551.
76. Ueda S, Matsuoka H, Miyazaki H, Usui M, Okuda S, Imaizumi T. Tetrahydrobiopterin restores endothelial function in long-term smokers. *J Am Coll Cardiol*. 2000;35:71–75.
77. Li H, Hortmann M, Daiber A, Oelze M, Ostad MA, Schwarz PM, Xu H, Xia N, Kleschyov AL, Mang C, Warnholtz A, Münzel T, Förstermann U. Cyclooxygenase 2-selective and nonselective nonsteroidal anti-inflammatory drugs induce oxidative stress by up-regulating vascular NADPH oxidases. *J Pharmacol Exp Ther*. 2008;326:745–753. doi: 10.1124/jpet.108.139030.
78. Goncharov NV, Avdonin PV, Nadeev AD, Zharkikh IL, Jenkins RO. Reactive oxygen species in pathogenesis of atherosclerosis. *Curr Pharm Des*. 2015;21:1134–1146.
79. McClelland S, Gawaz M, Kennerknecht E, Konrad CS, Sauer S, Schuerzinger K, Massberg S, Fitzgerald DJ, Belton O. Contribution of cyclooxygenase-1 to thromboxane formation, platelet-vessel wall interactions and atherosclerosis in the ApoE null mouse. *Atherosclerosis*. 2009;202:84–91. doi: 10.1016/j.atherosclerosis.2008.04.016.
80. Praticò D, Tillmann C, Zhang ZB, Li H, FitzGerald GA. Acceleration of atherogenesis by COX-1-dependent prostanoid formation in low density lipoprotein receptor knockout mice. *Proc Natl Acad Sci U S A*. 2001;98:3358–3363. doi: 10.1073/pnas.061607398.
81. Hui Y, Ricciotti E, Crichton I, Yu Z, Wang D, Stubbe J, Wang M, Puré E, FitzGerald GA. Targeted deletions of cyclooxygenase-2 and atherogenesis in mice. *Circulation*. 2010;121:2654–2660. doi: 10.1161/CIRCULATIONAHA.109.910687.
82. Tang SY, Monslow J, Todd L, Lawson J, Puré E, FitzGerald GA. Cyclooxygenase-2 in endothelial and vascular smooth muscle cells restrains atherogenesis in hyperlipidemic mice. *Circulation*. 2014;129:1761–1769. doi: 10.1161/CIRCULATIONAHA.113.007913.
83. Kirkby NS, Lundberg MH, Wright WR, Warner TD, Paul-Clark MJ, Mitchell JA. COX-2 protects against atherosclerosis independently of local vascular prostacyclin: identification of COX-2 associated pathways implicate Rgl1 and lymphocyte networks. *PLoS One*. 2014;9:e98165. doi: 10.1371/journal.pone.0098165.
84. Faraci FM, Didion SP. Vascular protection: superoxide dismutase isoforms in the vessel wall. *Arterioscler Thromb Vasc Biol*. 2004;24:1367–1373. doi: 10.1161/01.ATV.0000133604.20182.cf.
85. Fukai T, Ushio-Fukai M. Superoxide dismutases: role in redox signaling, vascular function, and diseases. *Antioxid Redox Signal*. 2011;15:1583–1606. doi: 10.1089/ars.2011.3999.
86. Tribble DL, Gong EL, Leeuwenburgh C, Heinecke JW, Carlson EL, Verstuyft JG, Epstein CJ. Fatty streak formation in fat-fed mice expressing human copper-zinc superoxide dismutase. *Arterioscler Thromb Vasc Biol*. 1997;17:1734–1740.
87. Nelson SK, Bose SK, McCord JM. The toxicity of high-dose superoxide dismutase suggests that superoxide can both initiate and terminate lipid peroxidation in the reperfused heart. *Free Radic Biol Med*. 1994;16:195–200.
88. Yang H, Roberts LJ, Shi MJ, Zhou LC, Ballard BR, Richardson A, Guo ZM. Retardation of atherosclerosis by overexpression of catalase or both Cu/Zn-superoxide dismutase and catalase in mice lacking apolipoprotein E. *Circ Res*. 2004;95:1075–1081. doi: 10.1161/01.RES.0000149564.49410.0d.
89. Strålin P, Karlsson K, Johansson BO, Marklund SL. The interstitium of the human arterial wall contains very large amounts of extracellular superoxide dismutase. *Arterioscler Thromb Vasc Biol*. 1995;15:2032–2036.
90. Sentman ML, Brännström T, Westerlund S, Laukkanen MO, Ylä-Herttuala S, Basu S, Marklund SL. Extracellular superoxide dismutase deficiency and atherosclerosis in mice. *Arterioscler Thromb Vasc Biol*. 2001;21:1477–1482.
91. Tribble DL, Barcellos-Hoff MH, Chu BM, Gong EL. Ionizing radiation accelerates aortic lesion formation in fat-fed mice via SOD-inhibitable processes. *Arterioscler Thromb Vasc Biol*. 1999;19:1387–1392.
92. Yang H, Zhou L, Wang Z, Roberts LJ 2nd, Lin X, Zhao Y, Guo Z. Overexpression of antioxidant enzymes in ApoE-deficient mice suppresses benzo(a)pyrene-accelerated atherosclerosis. *Atherosclerosis*. 2009;207:51–58. doi: 10.1016/j.atherosclerosis.2009.03.052.
93. de Haan JB, Witting PK, Stefanovic N, Pete J, Daskalakis M, Kola I, Stocker R, Smolich JJ. Lack of the antioxidant glutathione peroxidase-1 does not increase atherosclerosis in C57BL/J6 mice fed a high-fat diet. *J Lipid Res*. 2006;47:1157–1167. doi: 10.1194/jlr.M500377-JLR200.
94. Torzewski M, Ochsenhirt V, Kleschyov AL, Oelze M, Daiber A, Li H, Rossmann H, Tsimikas S, Reifenberg K, Cheng F, Lehr HA, Blankenberg S, Förstermann U, Münzel T, Lackner KJ. Deficiency of glutathione peroxidase-1 accelerates the progression of atherosclerosis in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*. 2007;27:850–857. doi: 10.1161/01.ATV.0000258809.47285.07.
95. Lewis P, Stefanovic N, Pete J, Calkin AC, Giunti S, Thallas-Bonke V, Jandeleit-Dahm KA, Allen TJ, Kola I, Cooper ME, de Haan JB. Lack of the antioxidant enzyme glutathione peroxidase-1 accelerates atherosclerosis in diabetic apolipoprotein E-deficient mice. *Circulation*. 2007;115:2178–2187. doi: 10.1161/CIRCULATIONAHA.106.664250.
96. Guo Z, Ran Q, Roberts LJ 2nd, Zhou L, Richardson A, Sharan C, Wu D, Yang H. Suppression of atherogenesis by overexpression of glutathione peroxidase-4 in apolipoprotein E-deficient mice. *Free Radic Biol Med*. 2008;44:343–352. doi: 10.1016/j.freeradbiomed.2007.09.009.
97. Tward A, Xia YR, Wang XP, Shi YS, Park C, Castellani LW, Lusis AJ, Shih DM. Decreased atherosclerotic lesion formation in human serum paraoxonase transgenic mice. *Circulation*. 2002;106:484–490.
98. Shih DM, Gu L, Xia YR, Navab M, Li WF, Hama S, Castellani LW, Furlong CE, Costa LG, Fogelman AM, Lusis AJ. Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. *Nature*. 1998;394:284–287. doi: 10.1038/28406.
99. Ng CJ, Hama SY, Bourquard N, Navab M, Reddy ST. Adenovirus mediated expression of human paraoxonase 2 protects against the development of atherosclerosis in apolipoprotein E-deficient mice. *Mol Genet Metab*. 2006;89:368–373. doi: 10.1016/j.ymgme.2006.07.004.
100. Ng CJ, Bourquard N, Grijalva V, Hama S, Shih DM, Navab M, Fogelman AM, Lusis AJ, Young S, Reddy ST. Paraoxonase-2 deficiency aggravates atherosclerosis in mice despite lower apolipoprotein-B-containing lipoproteins: anti-atherogenic role for paraoxonase-2. *J Biol Chem*. 2006;281:29491–29500. doi: 10.1074/jbc.M605379200.
101. Shih DM, Xia YR, Wang XP, Wang SS, Bourquard N, Fogelman AM, Lusis AJ, Reddy ST. Decreased obesity and atherosclerosis in human paraoxonase 3 transgenic mice. *Circ Res*. 2007;100:1200–1207. doi: 10.1161/01.RES.0000264499.48737.69.
102. Zhang H, Luo Y, Zhang W, He Y, Dai S, Zhang R, Huang Y, Bernatchez P, Giordano FJ, Shadel G, Sessa WC, Min W. Endothelial-specific expression of mitochondrial thioredoxin improves endothelial cell function and reduces atherosclerotic lesions. *Am J Pathol*. 2007;170:1108–1120. doi: 10.2353/ajpath.2007.060960.
103. Asmis R, Begley JG. Oxidized LDL promotes peroxide-mediated mitochondrial dysfunction and cell death in human macrophages: a caspase-3-independent pathway. *Circ Res*. 2003;92:e20–e29.
104. Lubos E, Loscalzo J, Handy DE. Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. *Antioxid Redox Signal*. 2011;15:1957–1997. doi: 10.1089/ars.2010.3586.
105. Blankenberg S, Rupprecht HJ, Bickel C, Torzewski M, Hafner G, Tiret L, Smieja M, Cambien F, Meyer J, Lackner KJ; AtheroGene Investigators. Glutathione peroxidase 1 activity and cardiovascular events in patients with coronary artery disease. *N Engl J Med*. 2003;349:1605–1613. doi: 10.1056/NEJMoa030535.
106. Cheng F, Torzewski M, Degreif A, Rossmann H, Canisius A, Lackner KJ. Impact of glutathione peroxidase-1 deficiency on macrophage foam cell formation and proliferation: implications for atherogenesis. *PLoS One*. 2013;8:e72063. doi: 10.1371/journal.pone.0072063.
107. Chew P, Yuen DY, Koh P, Stefanovic N, Febbraio MA, Kola I, Cooper ME, de Haan JB. Site-specific antiatherogenic effect of the antioxidant ebselen in the diabetic apolipoprotein E-deficient mouse. *Arterioscler Thromb Vasc Biol*. 2009;29:823–830. doi: 10.1161/ATVBAHA.109.186619.
108. Imai H, Nakagawa Y. Biological significance of phospholipid hydroperoxide glutathione peroxidase (PHGPx, GPx4) in mammalian cells. *Free Radic Biol Med*. 2003;34:145–169.
109. Draganov DI, Teiber JF, Speelman A, Osawa Y, Sunahara R, La Du BN. Human paraoxonases (PON1, PON2, and PON3) are

lactonases with overlapping and distinct substrate specificities. *J Lipid Res*. 2005;46:1239–1247. doi: 10.1194/jlr.M400511-JLR200.
110. Mackness M, Mackness B. Targeting paraoxonase-1 in atherosclerosis. *Expert Opin Ther Targets*. 2013;17:829–837. doi: 10.1517/14728222.2013.790367.
111. Aviram M, Vaya J. Paraoxonase 1 activities, regulation, and interactions with atherosclerotic lesion. *Curr Opin Lipidol*. 2013;24:339–344. doi: 10.1097/MOL.0b013e32835ffcfd.
112. Costa LG, Cole TB, Furlong CE. Paraoxonase (PON1): from toxicology to cardiovascular medicine. *Acta Biomed*. 2005;76 Suppl 2:50–57.
113. Mackness MI, Arrol S, Abbott C, Durrington PN. Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. *Atherosclerosis*. 1993;104:129–135.
114. Ng CJ, Wadleigh DJ, Gangopadhyay A, Hama S, Grijalva VR, Navab M, Fogelman AM, Reddy ST. Paraoxonase-2 is a ubiquitously expressed protein with antioxidant properties and is capable of preventing cell-mediated oxidative modification of low density lipoprotein. *J Biol Chem*. 2001;276:44444–44449. doi: 10.1074/jbc.M105660200.
115. Witte I, Foerstermann U, Devarajan A, Reddy ST, Horke S. Protectors or traitors: the roles of PON2 and PON3 in atherosclerosis and cancer. *J Lipids*. 2012;2012:342806. doi: 10.1155/2012/342806.
116. Horke S, Witte I, Wilgenbus P, Krüger M, Strand D, Förstermann U. Paraoxonase-2 reduces oxidative stress in vascular cells and decreases endoplasmic reticulum stress-induced caspase activation. *Circulation*. 2007;115:2055–2064. doi: 10.1161/CIRCULATIONAHA.106.681700.
117. Hagmann H, Kuczkowski A, Ruehl M, Lamkemeyer T, Brodesser S, Horke S, Dryer S, Schermer B, Benzing T, Brinkkoetter PT. Breaking the chain at the membrane: paraoxonase 2 counteracts lipid peroxidation at the plasma membrane. *FASEB J*. 2014;28:1769–1779. doi: 10.1096/fj.13-240309.
118. Fortunato G, Di Taranto MD, Bracale UM, Del Guercio L, Carbone F, Mazzaccara C, Morgante A, D'Armiento FP, D'Armiento M, Porcellini M, Sacchetti L, Bracale G, Salvatore F. Decreased paraoxonase-2 expression in human carotids during the progression of atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2008;28:594–600. doi: 10.1161/ATVBAHA.107.154658.
119. Draganov DI, Stetson PL, Watson CE, Billecke SS, La Du BN. Rabbit serum paraoxonase 3 (PON3) is a high density lipoprotein-associated lactonase and protects low density lipoprotein against oxidation. *J Biol Chem*. 2000;275:33435–33442. doi: 10.1074/jbc.M004543200.
120. Schweikert EM, Devarajan A, Witte I, Wilgenbus P, Amort J, Förstermann U, Shabazian A, Grijalva V, Shih DM, Farias-Eisner R, Teiber JF, Reddy ST, Horke S. PON3 is upregulated in cancer tissues and protects against mitochondrial superoxide-mediated cell death. *Cell Death Differ*. 2012;19:1549–1560. doi: 10.1038/cdd.2012.35.
121. Altenhöfer S, Witte I, Teiber JF, Wilgenbus P, Pautz A, Li H, Daiber A, Witan H, Clement AM, Förstermann U, Horke S. One enzyme, two functions: PON2 prevents mitochondrial superoxide formation and apoptosis independent from its lactonase activity. *J Biol Chem*. 2010;285:24398–24403. doi: 10.1074/jbc.M110.118604.
122. Marsillach J, Camps J, Beltran-Debón R, Rull A, Aragones G, Maestre-Martínez C, Sabench F, Hernández M, Castillo DD, Joven J, Mackness M, Mackness B. Immunohistochemical analysis of paraoxonases-1 and 3 in human atheromatous plaques. *Eur J Clin Invest*. 2011;41:308–314. doi: 10.1111/j.1365-2362.2010.02411.x.
123. Hanschmann EM, Godoy JR, Berndt C, Hudemann C, Lillig CH. Thioredoxins, glutaredoxins, and peroxiredoxins–molecular mechanisms and health significance: from cofactors to antioxidants to redox signaling. *Antioxid Redox Signal*. 2013;19:1539–1605. doi: 10.1089/ars.2012.4599.
124. Huang Q, Zhou HJ, Zhang H, Huang Y, Hinojosa-Kirschenbaum F, Fan P, Yao L, Belardinelli L, Tellides G, Giordano FJ, Budas GR, Min W. Thioredoxin-2 inhibits mitochondrial reactive oxygen species generation and apoptosis stress kinase-1 activity to maintain cardiac function. *Circulation*. 2015;131:1082–1097. doi: 10.1161/CIRCULATIONAHA.114.012725.
125. Kirsch J, Schneider H, Pagel JI, et al. Endothelial Dysfunction, and A Prothrombotic, Proinflammatory Phenotype Is Caused by Loss of Mitochondrial Thioredoxin Reductase in Endothelium. *Arterioscler Thromb Vasc Biol*. 2016;36:1891–1899. doi: 10.1161/ATVBAHA.116.307843.
126. Liu VW, Huang PL. Cardiovascular roles of nitric oxide: a review of insights from nitric oxide synthase gene disrupted mice. *Cardiovasc Res*. 2008;77:19–29. doi: 10.1016/j.cardiores.2007.06.024.
127. Schwarz PM, Kleinert H, Förstermann U. Potential functional significance of brain-type and muscle-type nitric oxide synthase I expressed in adventitia and media of rat aorta. *Arterioscler Thromb Vasc Biol*. 1999;19:2584–2590.
128. Tsutsui M. Neuronal nitric oxide synthase as a novel anti-atherogenic factor. *J Atheroscler Thromb*. 2004;11:41–48.
129. Schödel J, Padmapriya P, Marx A, Huang PL, Ertl G, Kuhlencordt PJ. Expression of neuronal nitric oxide synthase splice variants in atherosclerotic plaques of apoE knockout mice. *Atherosclerosis*. 2009;206:383–389. doi: 10.1016/j.atherosclerosis.2009.02.033.
130. Capettini LS, Cortes SF, Silva JF, Alvarez-Leite JI, Lemos VS. Decreased production of neuronal NOS-derived hydrogen peroxide contributes to endothelial dysfunction in atherosclerosis. *Br J Pharmacol*. 2011;164:1738–1748. doi: 10.1111/j.1476-5381.2011.01500.x.
131. Benkhoff S, Loot AE, Pierson I, Sturza A, Kohlstedt K, Fleming I, Shimokawa H, Grisk O, Brandes RP, Schröder K. Leptin potentiates endothelium-dependent relaxation by inducing endothelial expression of neuronal NO synthase. *Arterioscler Thromb Vasc Biol*. 2012;32:1605–1612. doi: 10.1161/ATVBAHA.112.251140.
132. Sanz MJ, Hickey MJ, Johnston B, McCafferty DM, Raharjo E, Huang PL, Kubes P. Neuronal nitric oxide synthase (NOS) regulates leukocyte-endothelial cell interactions in endothelial NOS deficient mice. *Br J Pharmacol*. 2001;134:305–312. doi: 10.1038/sj.bjp.0704234.
133. Talukder MA, Fujiki T, Morikawa K, Motoishi M, Kubota H, Morishita T, Tsutsui M, Takeshita A, Shimokawa H. Up-regulated neuronal nitric oxide synthase compensates coronary flow response to bradykinin in endothelial nitric oxide synthase-deficient mice. *J Cardiovasc Pharmacol*. 2004;44:437–445.
134. Seddon MD, Chowienczyk PJ, Brett SE, Casadei B, Shah AM. Neuronal nitric oxide synthase regulates basal microvascular tone in humans in vivo. *Circulation*. 2008;117:1991–1996. doi: 10.1161/CIRCULATIONAHA.107.744540.
135. Seddon M, Melikian N, Dworakowski R, Shabeeh H, Jiang B, Byrne J, Casadei B, Chowienczyk P, Shah AM. Effects of neuronal nitric oxide synthase on human coronary artery diameter and blood flow in vivo. *Circulation*. 2009;119:2656–2662. doi: 10.1161/CIRCULATIONAHA.108.822205.
136. Morishita T, Tsutsui M, Shimokawa H, Horiuchi M, Tanimoto A, Suda O, Tasaki H, Huang PL, Sasaguri Y, Yanagihara N, Nakashima Y. Vasculoprotective roles of neuronal nitric oxide synthase. *FASEB J*. 2002;16:1994–1996. doi: 10.1096/fj.02-0155fje.
137. Kuhlencordt PJ, Hötten S, Schödel J, Rützel S, Hu K, Widder J, Marx A, Huang PL, Ertl G. Atheroprotective effects of neuronal nitric oxide synthase in apolipoprotein e knockout mice. *Arterioscler Thromb Vasc Biol*. 2006;26:1539–1544. doi: 10.1161/01.ATV.0000223143.88128.19.
138. Kuhlencordt PJ, Chen J, Han F, Astern J, Huang PL. Genetic deficiency of inducible nitric oxide synthase reduces atherosclerosis and lowers plasma lipid peroxides in apolipoprotein E-knockout mice. *Circulation*. 2001;103:3099–3104.
139. Kuhlencordt PJ, Gyurko R, Han F, Scherrer-Crosbie M, Aretz TH, Hajjar R, Picard MH, Huang PL. Accelerated atherosclerosis, aortic aneurysm formation, and ischemic heart disease in apolipoprotein E/endothelial nitric oxide synthase double-knockout mice. *Circulation*. 2001;104:448–454.
140. Chen J, Kuhlencordt PJ, Astern J, Gyurko R, Huang PL. Hypertension does not account for the accelerated atherosclerosis and development of aneurysms in male apolipoprotein e/endothelial nitric oxide synthase double knockout mice. *Circulation*. 2001;104:2391–2394.
141. Ponnuswamy P, Schröttle A, Ostermeier E, Grüner S, Huang PL, Ertl G, Hoffmann U, Nieswandt B, Kuhlencordt PJ. eNOS protects from atherosclerosis despite relevant superoxide production by the enzyme in apoE mice. *PLoS One*. 2012;7:e30193. doi: 10.1371/journal.pone.0030193.
142. Ozaki M, Kawashima S, Yamashita T, Hirase T, Namiki M, Inoue N, Hirata K, Yasui H, Sakurai H, Yoshida Y, Masada M, Yokoyama M. Overexpression of endothelial nitric oxide synthase accelerates atherosclerotic lesion formation in apoE-deficient mice. *J Clin Invest*. 2002;110:331–340. doi: 10.1172/JCI15215.
143. Takaya T, Hirata K, Yamashita T, Shinohara M, Sasaki N, Inoue N, Yada T, Goto M, Fukatsu A, Hayashi T, Alp NJ, Channon KM, Yokoyama M, Kawashima S. A specific role for eNOS-derived reactive oxygen species in atherosclerosis progression. *Arterioscler Thromb Vasc Biol*. 2007;27:1632–1637. doi: 10.1161/ATVBAHA.107.142182.
144. Pautz A, Art J, Hahn S, Nowag S, Voss C, Kleinert H. Regulation of the expression of inducible nitric oxide synthase. *Nitric Oxide*. 2010;23:75–93. doi: 10.1016/j.niox.2010.04.007.
145. Gunnett CA, Lund DD, McDowell AK, Faraci FM, Heistad DD. Mechanisms of inducible nitric oxide synthase-mediated vascular

dysfunction. *Arterioscler Thromb Vasc Biol*. 2005;25:1617–1622. doi: 10.1161/01.ATV.0000172626.00296.ba.
146. Xia Y, Zweier JL. Superoxide and peroxynitrite generation from inducible nitric oxide synthase in macrophages. *Proc Natl Acad Sci U S A*. 1997;94:6954–6958.
147. Marfella R, Di Filippo C, Esposito K, Nappo F, Piegari E, Cuzzocrea S, Berrino L, Rossi F, Giugliano D, D'Amico M. Absence of inducible nitric oxide synthase reduces myocardial damage during ischemia reperfusion in streptozotocin-induced hyperglycemic mice. *Diabetes*. 2004;53:454–462.
148. Virdis A, Colucci R, Fornai M, Blandizzi C, Duranti E, Pinto S, Bernardini N, Segnani C, Antonioli L, Taddei S, Salvetti A, Del Tacca M. Cyclooxygenase-2 inhibition improves vascular endothelial dysfunction in a rat model of endotoxic shock: role of inducible nitric-oxide synthase and oxidative stress. *J Pharmacol Exp Ther*. 2005;312:945–953. doi: 10.1124/jpet.104.077644.
149. Depre C, Havaux X, Renkin J, Vanoverschelde JL, Wijns W. Expression of inducible nitric oxide synthase in human coronary atherosclerotic plaque. *Cardiovasc Res*. 1999;41:465–472.
150. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev*. 2007;87:315–424. doi: 10.1152/physrev.00029.2006.
151. Liaudet L, Rosenblatt-Velin N, Pacher P. Role of peroxynitrite in the cardiovascular dysfunction of septic shock. *Curr Vasc Pharmacol*. 2013;11:196–207.
152. Wilcox JN, Subramanian RR, Sundell CL, Tracey WR, Pollock JS, Harrison DG, Marsden PA. Expression of multiple isoforms of nitric oxide synthase in normal and atherosclerotic vessels. *Arterioscler Thromb Vasc Biol*. 1997;17:2479–2488.
153. Baker CS, Hall RJ, Evans TJ, Pomerance A, Maclouf J, Creminon C, Yacoub MH, Polak JM. Cyclooxygenase-2 is widely expressed in atherosclerotic lesions affecting native and transplanted human coronary arteries and colocalizes with inducible nitric oxide synthase and nitrotyrosine particularly in macrophages. *Arterioscler Thromb Vasc Biol*. 1999;19:646–655.
154. Hare JM, Stamler JS. NO/redox disequilibrium in the failing heart and cardiovascular system. *J Clin Invest*. 2005;115:509–517. doi: 10.1172/JCI24459.
155. Xia N, Li H. The role of perivascular adipose tissue in obesity-induced vascular dysfunction [published online ahead of print October 20, 2016]. *Br J Pharmacol*. doi: 10.1111/bph.13650. http://onlinelibrary.wiley.com/doi/10.1111/bph.13650/abstract.
156. Xia N, Horke S, Habermeier A, Closs EI, Reifenberg G, Gericke A, Mikhed Y, Münzel T, Daiber A, Förstermann U, Li H. Uncoupling of endothelial nitric oxide synthase in perivascular adipose tissue of diet-induced obese mice. *Arterioscler Thromb Vasc Biol*. 2016;36:78–85. doi: 10.1161/ATVBAHA.115.306263.
157. Bentzon JF, Falk E. Atherosclerotic lesions in mouse and man: is it the same disease? *Curr Opin Lipidol*. 2010;21:434–440. doi: 10.1097/MOL.0b013e32833ded6a.
158. Gleissner CA. Translational atherosclerosis research: From experimental models to coronary artery disease in humans. *Atherosclerosis*. 2016;248:110–116. doi: 10.1016/j.atherosclerosis.2016.03.013.
159. Matoba T, Sato K, Egashira K. Mouse models of plaque rupture. *Curr Opin Lipidol*. 2013;24:419–425. doi: 10.1097/MOL.0b013e3283646e4d.
160. Sato K, Nakano K, Katsuki S, Matoba T, Osada K, Sawamura T, Sunagawa K, Egashira K. Dietary cholesterol oxidation products accelerate plaque destabilization and rupture associated with monocyte infiltration/activation via the MCP-1-CCR2 pathway in mouse brachiocephalic arteries: therapeutic effects of ezetimibe. *J Atheroscler Thromb*. 2012;19:986–998.
161. Van der Donckt C, Van Herck JL, Schrijvers DM, et al. Elastin fragmentation in atherosclerotic mice leads to intraplaque neovascularization, plaque rupture, myocardial infarction, stroke, and sudden death. *Eur Heart J*. 2015;36:1049–1058. doi: 10.1093/eurheartj/ehu041.
162. Getz GS, Reardon CA. Do the Apoe-/- and Ldlr-/- Mice Yield the Same Insight on Atherogenesis? *Arterioscler Thromb Vasc Biol*. 2016;36:1734–1741. doi: 10.1161/ATVBAHA.116.306874.
163. Miller AA, De Silva TM, Jackman KA, Sobey CG. Effect of gender and sex hormones on vascular oxidative stress. *Clin Exp Pharmacol Physiol*. 2007;34:1037–1043. doi: 10.1111/j.1440-1681.2007.04732.x.
164. Joakimsen O, Bonaa KH, Stensland-Bugge E, Jacobsen BK. Age and sex differences in the distribution and ultrasound morphology of carotid atherosclerosis: the Tromsø Study. *Arterioscler Thromb Vasc Biol*. 1999;19:3007–3013.
165. Tsuda M, Iwai M, Li JM, Li HS, Min LJ, Ide A, Okumura M, Suzuki J, Mogi M, Suzuki H, Horiuchi M. Inhibitory effects of AT1 receptor blocker, olmesartan, and estrogen on atherosclerosis via anti-oxidative stress. *Hypertension*. 2005;45:545–551. doi: 10.1161/01.HYP.0000157409.88971.fc.
166. Miller AA, Drummond GR, Mast AE, Schmidt HH, Sobey CG. Effect of gender on NADPH-oxidase activity, expression, and function in the cerebral circulation: role of estrogen. *Stroke*. 2007;38:2142–2149. doi: 10.1161/STROKEAHA.106.477406.
167. Laughlin MH, Welshons WV, Sturek M, Rush JW, Turk JR, Taylor JA, Judy BM, Henderson KK, Ganjam VK. Gender, exercise training, and eNOS expression in porcine skeletal muscle arteries. *J Appl Physiol (1985)*. 2003;95:250–264. doi: 10.1152/japplphysiol.00061.2003.
168. Kleinert H, Wallerath T, Euchenhofer C, Ihrig-Biedert I, Li H, Förstermann U. Estrogens increase transcription of the human endothelial NO synthase gene: analysis of the transcription factors involved. *Hypertension*. 1998;31:582–588.
169. Nikpay M, Goel A, Won HH, et al; CARDIoGRAMplusC4D Consortium. A comprehensive 1,000 genomes-based genome-wide association meta-analysis of coronary artery disease. *Nat Genet*. 2015;47:1121–1130. doi: 10.1038/ng.3396.
170. Nurnberg ST, Zhang H, Hand NJ, Bauer RC, Saleheen D, Reilly MP, Rader DJ. From loci to biology: functional genomics of genome-wide association for coronary disease. *Circ Res*. 2016;118:586–606. doi: 10.1161/CIRCRESAHA.115.306464.
171. Inoue N, Kawashima S, Kanazawa K, Yamada S, Akita H, Yokoyama M. Polymorphism of the NADH/NADPH oxidase p22 phox gene in patients with coronary artery disease. *Circulation*. 1998;97:135–137.
172. Shimokata K, Yamada Y, Kondo T, Ichihara S, Izawa H, Nagata K, Murohara T, Ohno M, Yokota M. Association of gene polymorphisms with coronary artery disease in individuals with or without nonfamilial hypercholesterolemia. *Atherosclerosis*. 2004;172:167–173.
173. Arca M, Conti B, Montali A, Pignatelli P, Campagna F, Barillà F, Tanzilli G, Verna R, Vestri A, Gaudio C, Violi F. C242T polymorphism of NADPH oxidase p22phox and recurrence of cardiovascular events in coronary artery disease. *Arterioscler Thromb Vasc Biol*. 2008;28:752–757. doi: 10.1161/ATVBAHA.107.154823.
174. Cahilly C, Ballantyne CM, Lim DS, Gotto A, Marian AJ. A variant of p22(phox), involved in generation of reactive oxygen species in the vessel wall, is associated with progression of coronary atherosclerosis. *Circ Res*. 2000;86:391–395.
175. Andreassi MG, Adlerstein D, Carpeggiani C, Shehi E, Fantinato S, Ghezzi E, Botto N, Coceani M, L'abbate A. Individual and summed effects of high-risk genetic polymorphisms on recurrent cardiovascular events following ischemic heart disease. *Atherosclerosis*. 2012;223:409–415. doi: 10.1016/j.atherosclerosis.2012.05.029.
176. Neves AL, Mohammedi K, Emery N, Roussel R, Fumeron F, Marre M, Velho G. Allelic variations in superoxide dismutase-1 (SOD1) gene and renal and cardiovascular morbidity and mortality in type 2 diabetic subjects. *Mol Genet Metab*. 2012;106:359–365. doi: 10.1016/j.ymgme.2012.04.023.
177. Kakko S, Päivänsalo M, Koistinen P, Kesäniemi YA, Kinnula VL, Savolainen MJ. The signal sequence polymorphism of the MnSOD gene is associated with the degree of carotid atherosclerosis. *Atherosclerosis*. 2003;168:147–152.
178. Fujimoto H, Taguchi J, Imai Y, Ayabe S, Hashimoto H, Kobayashi H, Ogasawara K, Aizawa T, Yamakado M, Nagai R, Ohno M. Manganese superoxide dismutase polymorphism affects the oxidized low-density lipoprotein-induced apoptosis of macrophages and coronary artery disease. *Eur Heart J*. 2008;29:1267–1274. doi: 10.1093/eurheartj/ehm500.
179. Möllsten A, Jorsal A, Lajer M, Vionnet N, Tarnow L. The V16A polymorphism in SOD2 is associated with increased risk of diabetic nephropathy and cardiovascular disease in type 1 diabetes. *Diabetologia*. 2009;52:2590–2593. doi: 10.1007/s00125-009-1550-1.
180. Marklund SL, Nilsson P, Israelsson K, Schampi I, Peltonen M, Asplund K. Two variants of extracellular-superoxide dismutase: relationship to cardiovascular risk factors in an unselected middle-aged population. *J Intern Med*. 1997;242:5–14.
181. Juul K, Tybjaerg-Hansen A, Marklund S, Heegaard NH, Steffensen R, Sillesen H, Jensen G, Nordestgaard BG. Genetically reduced antioxidative protection and increased ischemic heart disease risk: The Copenhagen City Heart Study. *Circulation*. 2004;109:59–65. doi: 10.1161/01.CIR.0000105720.28086.6C.
182. Hamanishi T, Furuta H, Kato H, Doi A, Tamai M, Shimomura H, Sakagashira S, Nishi M, Sasaki H, Sanke T, Nanjo K. Functional

variants in the glutathione peroxidase-1 (GPx-1) gene are associated with increased intima-media thickness of carotid arteries and risk of macrovascular diseases in japanese type 2 diabetic patients. *Diabetes*. 2004;53:2455–2460.
183. Heslop CL, Tebbutt SJ, Podder M, Ruan J, Hill JS. Combined polymorphisms in oxidative stress genes predict coronary artery disease and oxidative stress in coronary angiography patients. *Ann Hum Genet*. 2012;76:435–447. doi: 10.1111/j.1469-1809.2012.00731.x.
184. Nemoto M, Nishimura R, Sasaki T, Hiki Y, Miyashita Y, Nishioka M, Fujimoto K, Sakuma T, Ohashi T, Fukuda K, Eto Y, Tajima N. Genetic association of glutathione peroxidase-1 with coronary artery calcification in type 2 diabetes: a case control study with multi-slice computed tomography. *Cardiovasc Diabetol*. 2007;6:23. doi: 10.1186/1475-2840-6-23.
185. Najafi M, Ghasemi H, Roustazadeh A, Farajollahi M. Lack of association between glutathione peroxidase1 (GPx1) activity, Pro198Leu polymorphism and stenosis of coronary arteries: A population-based prediction. *Meta Gene*. 2014;2:722–729. doi: 10.1016/j.mgene.2014.09.007.
186. Souiden Y, Mallouli H, Meskhi S, Chaabouni Y, Rebai A, Chéour F, Mahdouani K. MnSOD and GPx1 polymorphism relationship with coronary heart disease risk and severity. *Biol Res*. 2016;49:22. doi: 10.1186/s40659-016-0083-6.
187. Casas JP, Cavalleri GL, Bautista LE, Smeeth L, Humphries SE, Hingorani AD. Endothelial nitric oxide synthase gene polymorphisms and cardiovascular disease: a HuGE review. *Am J Epidemiol*. 2006;164:921–935. doi: 10.1093/aje/kwj302.
188. Casas JP, Bautista LE, Humphries SE, Hingorani AD. Endothelial nitric oxide synthase genotype and ischemic heart disease: meta-analysis of 26 studies involving 23028 subjects. *Circulation*. 2004;109:1359–1365. doi: 10.1161/01.CIR.0000121357.76910.A3.
189. Rai H, Parveen F, Kumar S, Kapoor A, Sinha N. Association of endothelial nitric oxide synthase gene polymorphisms with coronary artery disease: an updated meta-analysis and systematic review. *PLoS One*. 2014;9:e113363. doi: 10.1371/journal.pone.0113363.
190. Liu D, Jiang Z, Dai L, Zhang X, Yan C, Han Y. Association between the - 786T>C 1polymorphism in the promoter region of endothelial nitric oxide synthase (eNOS) and risk of coronary artery disease: a systematic review and meta-analysis. *Gene*. 2014;545:175–183. doi: 10.1016/j.gene.2013.09.099.
191. San José G, Fortuño A, Beloqui O, Díez J, Zalba G. NADPH oxidase CYBA polymorphisms, oxidative stress and cardiovascular diseases. *Clin Sci (Lond)*. 2008;114:173–182. doi: 10.1042/CS20070130.
192. Wyche KE, Wang SS, Griendling KK, Dikalov SI, Austin H, Rao S, Fink B, Harrison DG, Zafari AM. C242T CYBA polymorphism of the NADPH oxidase is associated with reduced respiratory burst in human neutrophils. *Hypertension*. 2004;43:1246–1251. doi: 10.1161/01.HYP.0000126579.50711.62.
193. Guzik TJ, West NE, Black E, McDonald D, Ratnatunga C, Pillai R, Channon KM. Functional effect of the C242T polymorphism in the NAD(P)H oxidase p22phox gene on vascular superoxide production in atherosclerosis. *Circulation*. 2000;102:1744–1747.
194. Wu Z, Lou Y, Jin W, Liu Y, Lu L, Chen Q, Xie Y, Lu G. Relationship of the p22phox (CYBA) gene polymorphism C242T with risk of coronary artery disease: a meta-analysis. *PLoS One*. 2013;8:e70885. doi: 10.1371/journal.pone.0070885.
195. Xu Q, Yuan F, Shen X, Wen H, Li W, Cheng B, Wu J. Polymorphisms of C242T and A640G in CYBA gene and the risk of coronary artery disease: a meta-analysis. *PLoS One*. 2014;9:e84251. doi: 10.1371/journal.pone.0084251.
196. Violi F, Sanguigni V, Carnevale R, et al. Hereditary deficiency of gp91(phox) is associated with enhanced arterial dilatation: results of a multicenter study. *Circulation*. 2009;120:1616–1622. doi: 10.1161/CIRCULATIONAHA.109.877191.
197. Loukogeorgakis SP, van den Berg MJ, Sofat R, Nitsch D, Charakida M, Haiyee B, de Groot E, MacAllister RJ, Kuijpers TW, Deanfield JE. Role of NADPH oxidase in endothelial ischemia/reperfusion injury in humans. *Circulation*. 2010;121:2310–2316. doi: 10.1161/CIRCULATIONAHA.108.814731.
198. Violi F, Pignatelli P, Pignata C, Plebani A, Rossi P, Sanguigni V, Carnevale R, Soresina A, Finocchi A, Cirillo E, Catasca E, Angelico F, Loffredo L. Reduced atherosclerotic burden in subjects with genetically determined low oxidative stress. *Arterioscler Thromb Vasc Biol*. 2013;33:406–412. doi: 10.1161/ATVBAHA.112.300438.
199. Sibley CT, Estwick T, Zavodni A, et al. Assessment of atherosclerosis in chronic granulomatous disease. *Circulation*. 2014;130:2031–2039. doi: 10.1161/CIRCULATIONAHA.113.006824.
200. Sorescu D, Weiss D, Lassègue B, Clempus RE, Szöcs K, Sorescu GP, Valppu L, Quinn MT, Lambeth JD, Vega JD, Taylor WR, Griendling KK. Superoxide production and expression of nox family proteins in human atherosclerosis. *Circulation*. 2002;105:1429–1435.
201. Mohammedi K, Bellili-Muñoz N, Marklund SL, Driss F, Le Nagard H, Patente TA, Fumeron F, Roussel R, Hadjadj S, Marre M, Velho G. Plasma extracellular superoxide dismutase concentration, allelic variations in the SOD3 gene and risk of myocardial infarction and all-cause mortality in people with type 1 and type 2 diabetes. *Cardiovasc Diabetol*. 2015;14:845. doi: 10.1186/s12933-014-0163-2.
202. Sandström J, Nilsson P, Karlsson K, Marklund SL. 10-fold increase in human plasma extracellular superoxide dismutase content caused by a mutation in heparin-binding domain. *J Biol Chem*. 1994;269:19163–19166.
203. Adachi T, Yamada H, Yamada Y, Morihara N, Yamazaki N, Murakami T, Futenma A, Kato K, Hirano K. Substitution of glycine for arginine-213 in extracellular-superoxide dismutase impairs affinity for heparin and endothelial cell surface. *Biochem J*. 1996;313 (Pt 1):235–239.
204. Chu Y, Alwahdani A, Iida S, Lund DD, Faraci FM, Heistad DD. Vascular effects of the human extracellular superoxide dismutase R213G variant. *Circulation*. 2005;112:1047–1053. doi: 10.1161/CIRCULATIONAHA.104.531251.
205. Hartney JM, Stidham T, Goldstrohm DA, et al. A common polymorphism in extracellular superoxide dismutase affects cardiopulmonary disease risk by altering protein distribution. *Circ Cardiovasc Genet*. 2014;7:659–666. doi: 10.1161/CIRCGENETICS.113.000504.
206. Ratnasinghe D, Tangrea JA, Andersen MR, Barrett MJ, Virtamo J, Taylor PR, Albanes D. Glutathione peroxidase codon 198 polymorphism variant increases lung cancer risk. *Cancer Res*. 2000;60:6381–6383.
207. Zhang JX, Wang ZM, Zhang JJ, Zhu LL, Gao XF, Chen SL. Association of glutathione peroxidase-1 (GPx-1) rs1050450 Pro198Leu and Pro197Leu polymorphisms with cardiovascular risk: a meta-analysis of observational studies. *J Geriatr Cardiol*. 2014;11:141–150. doi: 10.3969/j.issn.1671-5411.2014.02.003.
208. Forsberg L, de Faire U, Marklund SL, Andersson PM, Stegmayr B, Morgenstern R. Phenotype determination of a common Pro-Leu polymorphism in human glutathione peroxidase 1. *Blood Cells Mol Dis*. 2000;26:423–426. doi: 10.1006/bcmd.2000.0325.
209. Vecoli C. Endothelial nitric oxide synthase gene polymorphisms in cardiovascular disease. *Vitam Horm*. 2014;96:387–406. doi: 10.1016/B978-0-12-800254-4.00015-5.
210. Tesauro M, Thompson WC, Rogliani P, Qi L, Chaudhary PP, Moss J. Intracellular processing of endothelial nitric oxide synthase isoforms associated with differences in severity of cardiopulmonary diseases: cleavage of proteins with aspartate vs. glutamate at position 298. *Proc Natl Acad Sci U S A*. 2000;97:2832–2835.
211. Persu A, Stoenoiu MS, Messiaen T, Davila S, Robino C, El-Khattabi O, Mourad M, Horie S, Feron O, Balligand JL, Wattiez R, Pirson Y, Chauveau D, Lens XM, Devuyst O. Modifier effect of ENOS in autosomal dominant polycystic kidney disease. *Hum Mol Genet*. 2002;11:229–241.
212. Fairchild TA, Fulton D, Fontana JT, Gratton JP, McCabe TJ, Sessa WC. Acidic hydrolysis as a mechanism for the cleavage of the Glu(298)–>Asp variant of human endothelial nitric-oxide synthase. *J Biol Chem*. 2001;276:26674–26679. doi: 10.1074/jbc.M103647200.
213. McDonald DM, Alp NJ, Channon KM. Functional comparison of the endothelial nitric oxide synthase Glu298Asp polymorphic variants in human endothelial cells. *Pharmacogenetics*. 2004;14:831–839.
214. Joshi MS, Mineo C, Shaul PW, Bauer JA. Biochemical consequences of the NOS3 Glu298Asp variation in human endothelium: altered caveolar localization and impaired response to shear. *FASEB J*. 2007;21:2655–2663. doi: 10.1096/fj.06-7088com.
215. Hingorani AD, Liang CF, Fatibene J, Lyon A, Monteith S, Parsons A, Haydock S, Hopper RV, Stephens NG, O'Shaughnessy KM, Brown MJ. A common variant of the endothelial nitric oxide synthase (Glu298–>Asp) is a major risk factor for coronary artery disease in the UK. *Circulation*. 1999;100:1515–1520.
216. Wang XL, Sim AS, Badenhop RF, McCredie RM, Wilcken DE. A smoking-dependent risk of coronary artery disease associated with a polymorphism of the endothelial nitric oxide synthase gene. *Nat Med*. 1996;2:41–45.
217. Colombo MG, Paradossi U, Andreassi MG, Botto N, Manfredi S, Masetti S, Biagini A, Clerico A. Endothelial nitric oxide synthase gene polymorphisms and risk of coronary artery disease. *Clin Chem*. 2003;49:389–395.
218. Karvonen J, Kauma H, Kervinen K, Rantala M, Ikäheimo M, Päivänsalo M, Savolainen MJ, Kesäniemi YA. Endothelial nitric oxide synthase gene Glu298Asp polymorphism and blood pressure, left ventricular mass and

carotid artery atherosclerosis in a population-based cohort. *J Intern Med*. 2002;251:102–110.
219. Granath B, Taylor RR, van Bockxmeer FM, Mamotte CD. Lack of evidence for association between endothelial nitric oxide synthase gene polymorphisms and coronary artery disease in the Australian Caucasian population. *J Cardiovasc Risk*. 2001;8:235–241.
220. Matsuno K, Yamada H, Iwata K, Jin D, Katsuyama M, Matsuki M, Takai S, Yamanishi K, Miyazaki M, Matsubara H, Yabe-Nishimura C. Nox1 is involved in angiotensin II-mediated hypertension: a study in Nox1-deficient mice. *Circulation*. 2005;112:2677–2685. doi: 10.1161/CIRCULATIONAHA.105.573709.
221. Li H, Witte K, August M, Brausch I, Gödtel-Armbrust U, Habermeier A, Closs EI, Oelze M, Münzel T, Förstermann U. Reversal of endothelial nitric oxide synthase uncoupling and up-regulation of endothelial nitric oxide synthase expression lowers blood pressure in hypertensive rats. *J Am Coll Cardiol*. 2006;47:2536–2544. doi: 10.1016/j.jacc.2006.01.071.
222. Chalupsky K, Cai H. Endothelial dihydrofolate reductase: critical for nitric oxide bioavailability and role in angiotensin II uncoupling of endothelial nitric oxide synthase. *Proc Natl Acad Sci U S A*. 2005;102:9056–9061. doi: 10.1073/pnas.0409594102.
223. Demougeot C, Prigent-Tessier A, Bagnost T, André C, Guillaume Y, Bouhaddi M, Marie C, Berthelot A. Time course of vascular arginase expression and activity in spontaneously hypertensive rats. *Life Sci*. 2007;80:1128–1134. doi: 10.1016/j.lfs.2006.12.003.
224. Rodriguez S, Richert L, Berthelot A. Increased arginase activity in aorta of mineralocorticoid-salt hypertensive rats. *Clin Exp Hypertens*. 2000;22:75–85.
225. Johnson FK, Johnson RA, Peyton KJ, Durante W. Arginase inhibition restores arteriolar endothelial function in Dahl rats with salt-induced hypertension. *Am J Physiol Regul Integr Comp Physiol*. 2005;288:R1057–R1062. doi: 10.1152/ajpregu.00758.2004.
226. Galougahi KK, Liu CC, Gentile C, Kok C, Nunez A, Garcia A, Fry NA, Davies MJ, Hawkins CL, Rasmussen HH, Figtree GA. Glutathionylation mediates angiotensin II-induced eNOS uncoupling, amplifying NADPH oxidase-dependent endothelial dysfunction. *J Am Heart Assoc*. 2014;3:e000731. doi: 10.1161/JAHA.113.000731.
227. Pritchard KA Jr, Groszek L, Smalley DM, Sessa WC, Wu M, Villalon P, Wolin MS, Stemerman MB. Native low-density lipoprotein increases endothelial cell nitric oxide synthase generation of superoxide anion. *Circ Res*. 1995;77:510–518.
228. Stepp DW, Ou J, Ackerman AW, Welak S, Klick D, Pritchard KA Jr. Native LDL and minimally oxidized LDL differentially regulate superoxide anion in vascular endothelium in situ. *Am J Physiol Heart Circ Physiol*. 2002;283:H750–H759. doi: 10.1152/ajpheart.00029.2002.
229. Nickenig G, Bäumer AT, Temur Y, Kebben D, Jockenhövel F, Böhm M. Statin-sensitive dysregulated AT1 receptor function and density in hypercholesterolemic men. *Circulation*. 1999;100:2131–2134.
230. Ryoo S, Gupta G, Benjo A, et al. Endothelial arginase II: a novel target for the treatment of atherosclerosis. *Circ Res*. 2008;102:923–932. doi: 10.1161/CIRCRESAHA.107.169573.
231. Erdely A, Kepka-Lenhart D, Salmen-Muniz R, Chapman R, Hulderman T, Kashon M, Simeonova PP, Morris SM Jr. Arginase activities and global arginine bioavailability in wild-type and ApoE-deficient mice: responses to high fat and high cholesterol diets. *PLoS One*. 2010;5:e15253. doi: 10.1371/journal.pone.0015253.
232. Hayashi T, Esaki T, Sumi D, Mukherjee T, Iguchi A, Chaudhuri G. Modulating role of estradiol on arginase II expression in hyperlipidemic rabbits as an atheroprotective mechanism. *Proc Natl Acad Sci U S A*. 2006;103:10485–10490. doi: 10.1073/pnas.0603918103.
233. Vaisman BL, Andrews KL, Khong SM, Wood KC, Moore XL, Fu Y, Kepka-Lenhart DM, Morris SM Jr, Remaley AT, Chin-Dusting JP. Selective endothelial overexpression of arginase II induces endothelial dysfunction and hypertension and enhances atherosclerosis in mice. *PLoS One*. 2012;7:e39487. doi: 10.1371/journal.pone.0039487.
234. Feron O, Dessy C, Moniotte S, Desager JP, Balligand JL. Hypercholesterolemia decreases nitric oxide production by promoting the interaction of caveolin and endothelial nitric oxide synthase. *J Clin Invest*. 1999;103:897–905. doi: 10.1172/JCI4829.
235. Chavakis E, Dernbach E, Hermann C, Mondorf UF, Zeiher AM, Dimmeler S. Oxidized LDL inhibits vascular endothelial growth factor-induced endothelial cell migration by an inhibitory effect on the Akt/endothelial nitric oxide synthase pathway. *Circulation*. 2001;103:2102–2107.
236. Steffen Y, Jung T, Klotz LO, Schewe T, Grune T, Sies H. Protein modification elicited by oxidized low-density lipoprotein (LDL) in endothelial cells: protection by (-)-epicatechin. *Free Radic Biol Med*. 2007;42:955–970. doi: 10.1016/j.freeradbiomed.2006.12.024.
237. Jaimes EA, DeMaster EG, Tian RX, Raij L. Stable compounds of cigarette smoke induce endothelial superoxide anion production via NADPH oxidase activation. *Arterioscler Thromb Vasc Biol*. 2004;24:1031–1036. doi: 10.1161/01.ATV.0000127083.88549.58.
238. Csiszar A, Labinskyy N, Pinto JT, Ballabh P, Zhang H, Losonczy G, Pearson K, de Cabo R, Pacher P, Zhang C, Ungvari Z. Resveratrol induces mitochondrial biogenesis in endothelial cells. *Am J Physiol Heart Circ Physiol*. 2009;297:H13–H20. doi: 10.1152/ajpheart.00368.2009.
239. Steffen Y, Vuillaume G, Stolle K, Roewer K, Lietz M, Schueller J, Lebrun S, Wallerath T. Cigarette smoke and LDL cooperate in reducing nitric oxide bioavailability in endothelial cells via effects on both eNOS and NADPH oxidase. *Nitric Oxide*. 2012;27:176–184. doi: 10.1016/j.niox.2012.06.006.
240. Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res*. 2010;107:1058–1070. doi: 10.1161/CIRCRESAHA.110.223545.
241. Funk DM. Coagulation assays and anticoagulant monitoring. *Hematology Am Soc Hematol Educ Program*. 2012;2012:460–465. doi: 10.1182/asheducation-2012.1.460.
242. Hink U, Li H, Mollnau H, Oelze M, Matheis E, Hartmann M, Skatchkov M, Thaiss F, Stahl RA, Warnholtz A, Meinertz T, Griendling K, Harrison DG, Forstermann U, Munzel T. Mechanisms underlying endothelial dysfunction in diabetes mellitus. *Circ Res*. 2001;88:E14–E22.
243. Alp NJ, Mussa S, Khoo J, Cai S, Guzik T, Jefferson A, Goh N, Rockett KA, Channon KM. Tetrahydrobiopterin-dependent preservation of nitric oxide-mediated endothelial function in diabetes by targeted transgenic GTP-cyclohydrolase I overexpression. *J Clin Invest*. 2003;112:725–735. doi: 10.1172/JCI17786.
244. Faria AM, Papadimitriou A, Silva KC, Lopes de Faria JM, Lopes de Faria JB. Uncoupling endothelial nitric oxide synthase is ameliorated by green tea in experimental diabetes by re-establishing tetrahydrobiopterin levels. *Diabetes*. 2012;61:1838–1847. doi: 10.2337/db11-1241.
245. Wenzel P, Daiber A, Oelze M, Brandt M, Closs E, Xu J, Thum T, Bauersachs J, Ertl G, Zou MH, Förstermann U, Münzel T. Mechanisms underlying recoupling of eNOS by HMG-CoA reductase inhibition in a rat model of streptozotocin-induced diabetes mellitus. *Atherosclerosis*. 2008;198:65–76. doi: 10.1016/j.atherosclerosis.2007.10.003.
246. Xu J, Wu Y, Song P, Zhang M, Wang S, Zou MH. Proteasome-dependent degradation of guanosine 5'-triphosphate cyclohydrolase I causes tetrahydrobiopterin deficiency in diabetes mellitus. *Circulation*. 2007;116:944–953. doi: 10.1161/CIRCULATIONAHA.106.684795.
247. Schuhmacher S, Oelze M, Bollmann F, et al. Vascular dysfunction in experimental diabetes is improved by pentaerithrityl tetranitrate but not isosorbide-5-mononitrate therapy. *Diabetes*. 2011;60:2608–2616. doi: 10.2337/db10-1395.
248. Pannirselvam M, Simon V, Verma S, Anderson T, Triggle CR. Chronic oral supplementation with sepiapterin prevents endothelial dysfunction and oxidative stress in small mesenteric arteries from diabetic (db/db) mice. *Br J Pharmacol*. 2003;140:701–706. doi: 10.1038/sj.bjp.0705476.
249. Pannirselvam M, Verma S, Anderson TJ, Triggle CR. Cellular basis of endothelial dysfunction in small mesenteric arteries from spontaneously diabetic (db/db -/-) mice: role of decreased tetrahydrobiopterin bioavailability. *Br J Pharmacol*. 2002;136:255–263. doi: 10.1038/sj.bjp.0704683.
250. Shinozaki K, Nishio Y, Okamura T, Yoshida Y, Maegawa H, Kojima H, Masada M, Toda N, Kikkawa R, Kashiwagi A. Oral administration of tetrahydrobiopterin prevents endothelial dysfunction and vascular oxidative stress in the aortas of insulin-resistant rats. *Circ Res*. 2000;87:566–573.
251. Romero MJ, Platt DH, Tawfik HE, Labazi M, El-Remessy AB, Bartoli M, Caldwell RB, Caldwell RW. Diabetes-induced coronary vascular dysfunction involves increased arginase activity. *Circ Res*. 2008;102:95–102. doi: 10.1161/CIRCRESAHA.107.155028.
252. Yao L, Chandra S, Toque HA, Bhatta A, Rojas M, Caldwell RB, Caldwell RW. Prevention of diabetes-induced arginase activation and vascular dysfunction by Rho kinase (ROCK) knockout. *Cardiovasc Res*. 2013;97:509–519. doi: 10.1093/cvr/cvs371.
253. Giri H, Muthuramu I, Dhar M, Rathnakumar K, Ram U, Dixit M. Protein tyrosine phosphatase SHP2 mediates chronic insulin-induced endothelial inflammation. *Arterioscler Thromb Vasc Biol*. 2012;32:1943–1950. doi: 10.1161/ATVBAHA.111.239251.
254. Romero MJ, Iddings JA, Platt DH, Ali MI, Cederbaum SD, Stepp DW, Caldwell RB, Caldwell RW. Diabetes-induced vascular dysfunction involves arginase I. *Am J Physiol Heart Circ Physiol*. 2012;302:H159–H166. doi: 10.1152/ajpheart.00774.2011.

255. Toque HA, Tostes RC, Yao L, Xu Z, Webb RC, Caldwell RB, Caldwell RW. Arginase II deletion increases corpora cavernosa relaxation in diabetic mice. *J Sex Med*. 2011;8:722–733. doi: 10.1111/j.1743-6109.2010.02098.x.
256. Grönros J, Jung C, Lundberg JO, Cerrato R, Ostenson CG, Pernow J. Arginase inhibition restores in vivo coronary microvascular function in type 2 diabetic rats. *Am J Physiol Heart Circ Physiol*. 2011;300:H1174–H1181. doi: 10.1152/ajpheart.00560.2010.
257. Kashyap SR, Lara A, Zhang R, Park YM, DeFronzo RA. Insulin reduces plasma arginase activity in type 2 diabetic patients. *Diabetes Care*. 2008;31:134–139. doi: 10.2337/dc07-1198.
258. Beleznai T, Feher A, Spielvogel D, Lansman SL, Bagi Z. Arginase 1 contributes to diminished coronary arteriolar dilation in patients with diabetes. *Am J Physiol Heart Circ Physiol*. 2011;300:H777–H783. doi: 10.1152/ajpheart.00831.2010.
259. Shemyakin A, Kövamees O, Rafnsson A, Böhm F, Svenarud P, Settergren M, Jung C, Pernow J. Arginase inhibition improves endothelial function in patients with coronary artery disease and type 2 diabetes mellitus. *Circulation*. 2012;126:2943–2950. doi: 10.1161/CIRCULATIONAHA.112.140335.
260. Kwak BR, Bäck M, Bochaton-Piallat ML, et al. Biomechanical factors in atherosclerosis: mechanisms and clinical implications. *Eur Heart J*. 2014;35:3013–20, 3020a. doi: 10.1093/eurheartj/ehu353.
261. Cheng C, Tempel D, van Haperen R, van der Baan A, Grosveld F, Daemen MJ, Krams R, de Crom R. Atherosclerotic lesion size and vulnerability are determined by patterns of fluid shear stress. *Circulation*. 2006;113:2744–2753. doi: 10.1161/CIRCULATIONAHA.105.590018.
262. Cheng C, van Haperen R, de Waard M, van Damme LC, Tempel D, Hanemaaijer L, van Cappellen GW, Bos J, Slager CJ, Duncker DJ, van der Steen AF, de Crom R, Krams R. Shear stress affects the intracellular distribution of eNOS: direct demonstration by a novel in vivo technique. *Blood*. 2005;106:3691–3698. doi: 10.1182/blood-2005-06-2326.
263. Nam D, Ni CW, Rezvan A, Suo J, Budzyn K, Llanos A, Harrison D, Giddens D, Jo H. Partial carotid ligation is a model of acutely induced disturbed flow, leading to rapid endothelial dysfunction and atherosclerosis. *Am J Physiol Heart Circ Physiol*. 2009;297:H1535–H1543. doi: 10.1152/ajpheart.00510.2009.
264. Chen YC, Bui AV, Diesch J, Manasseh R, Hausding C, Rivera J, Haviv I, Agrotis A, Htun NM, Jowett J, Hagemeyer CE, Hannan RD, Bobik A, Peter K. A novel mouse model of atherosclerotic plaque instability for drug testing and mechanistic/therapeutic discoveries using gene and microRNA expression profiling. *Circ Res*. 2013;113:252–265. doi: 10.1161/CIRCRESAHA.113.301562.
265. Hopkins PN. Molecular biology of atherosclerosis. *Physiol Rev*. 2013;93:1317–1542. doi: 10.1152/physrev.00004.2012.
266. Chatzizisis YS, Coskun AU, Jonas M, Edelman ER, Feldman CL, Stone PH. Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: molecular, cellular, and vascular behavior. *J Am Coll Cardiol*. 2007;49:2379–2393. doi: 10.1016/j.jacc.2007.02.059.
267. Noris M, Morigi M, Donadelli R, Aiello S, Foppolo M, Todeschini M, Orisio S, Remuzzi G, Remuzzi A. Nitric oxide synthesis by cultured endothelial cells is modulated by flow conditions. *Circ Res*. 1995;76:536–543.
268. Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature*. 1999;399:601–605. doi: 10.1038/21224.
269. Gallis B, Corthals GL, Goodlett DR, Ueba H, Kim F, Presnell SR, Figeys D, Harrison DG, Berk BC, Aebersold R, Corson MA. Identification of flow-dependent endothelial nitric-oxide synthase phosphorylation sites by mass spectrometry and regulation of phosphorylation and nitric oxide production by the phosphatidylinositol 3-kinase inhibitor LY294002. *J Biol Chem*. 1999;274:30101–30108.
270. Fleming I. Molecular mechanisms underlying the activation of eNOS. *Pflugers Arch*. 2010;459:793–806. doi: 10.1007/s00424-009-0767-7.
271. Lam CF, Peterson TE, Richardson DM, Croatt AJ, d'Uscio LV, Nath KA, Katusic ZS. Increased blood flow causes coordinated upregulation of arterial eNOS and biosynthesis of tetrahydrobiopterin. *Am J Physiol Heart Circ Physiol*. 2006;290:H786–H793. doi: 10.1152/ajpheart.00759.2005.
272. Gambillara V, Chambaz C, Montorzi G, Roy S, Stergiopulos N, Silacci P. Plaque-prone hemodynamics impair endothelial function in pig carotid arteries. *Am J Physiol Heart Circ Physiol*. 2006;290:H2320–H2328. doi: 10.1152/ajpheart.00486.2005.
273. Ziegler T, Bouzourène K, Harrison VJ, Brunner HR, Hayoz D. Influence of oscillatory and unidirectional flow environments on the expression of endothelin and nitric oxide synthase in cultured endothelial cells. *Arterioscler Thromb Vasc Biol*. 1998;18:686–692.
274. De Keulenaer GW, Chappell DC, Ishizaka N, Nerem RM, Alexander RW, Griendling KK. Oscillatory and steady laminar shear stress differentially affect human endothelial redox state: role of a superoxide-producing NADH oxidase. *Circ Res*. 1998;82:1094–1101.
275. Hwang J, Ing MH, Salazar A, Lassègue B, Griendling K, Navab M, Sevanian A, Hsiai TK. Pulsatile versus oscillatory shear stress regulates NADPH oxidase subunit expression: implication for native LDL oxidation. *Circ Res*. 2003;93:1225–1232. doi: 10.1161/01.RES.0000104087.29395.66.
276. Harris CM, Sanders SA, Massey V. Role of the flavin midpoint potential and NAD binding in determining NAD versus oxygen reactivity of xanthine oxidoreductase. *J Biol Chem*. 1999;274:4561–4569.
277. Lima B, Forrester MT, Hess DT, Stamler JS. S-nitrosylation in cardiovascular signaling. *Circ Res*. 2010;106:633–646. doi: 10.1161/CIRCRESAHA.109.207381.
278. Selemidis S, Dusting GJ, Peshavariya H, Kemp-Harper BK, Drummond GR. Nitric oxide suppresses NADPH oxidase-dependent superoxide production by S-nitrosylation in human endothelial cells. *Cardiovasc Res*. 2007;75:349–358. doi: 10.1016/j.cardiores.2007.03.030.
279. Ravi K, Brennan LA, Levic S, Ross PA, Black SM. S-nitrosylation of endothelial nitric oxide synthase is associated with monomerization and decreased enzyme activity. *Proc Natl Acad Sci U S A*. 2004;101:2619–2624.
280. Liu L, Yan Y, Zeng M, Zhang J, Hanes MA, Ahearn G, McMahon TJ, Dickfeld T, Marshall HE, Que LG, Stamler JS. Essential roles of S-nitrosothiols in vascular homeostasis and endotoxic shock. *Cell*. 2004;116:617–628.
281. Song P, Zou MH. Redox regulation of endothelial cell fate. *Cell Mol Life Sci*. 2014;71:3219–3239. doi: 10.1007/s00018-014-1598-z.
282. King AL, Polhemus DJ, Bhushan S, et al. Hydrogen sulfide cytoprotective signaling is endothelial nitric oxide synthase-nitric oxide dependent. *Proc Natl Acad Sci U S A*. 2014;111:3182–3187. doi: 10.1073/pnas.1321871111.
283. Wang R, Szabo C, Ichinose F, Ahmed A, Whiteman M, Papapetropoulos A. The role of H2S bioavailability in endothelial dysfunction. *Trends Pharmacol Sci*. 2015;36:568–578. doi: 10.1016/j.tips.2015.05.007.
284. Yuan S, Patel RP, Kevil CG. Working with nitric oxide and hydrogen sulfide in biological systems. *Am J Physiol Lung Cell Mol Physiol*. 2015;308:L403–L415. doi: 10.1152/ajplung.00327.2014.
285. Altaany Z, Ju Y, Yang G, Wang R. The coordination of S-sulfhydration, S-nitrosylation, and phosphorylation of endothelial nitric oxide synthase by hydrogen sulfide. *Sci Signal*. 2014;7:ra87. doi: 10.1126/scisignal.2005478.
286. Chung HS, Wang SB, Venkatraman V, Murray CI, Van Eyk JE. Cysteine oxidative posttranslational modifications: emerging regulation in the cardiovascular system. *Circ Res*. 2013;112:382–392. doi: 10.1161/CIRCRESAHA.112.268680.
287. Brown DI, Griendling KK. Regulation of signal transduction by reactive oxygen species in the cardiovascular system. *Circ Res*. 2015;116:531–549. doi: 10.1161/CIRCRESAHA.116.303584.
288. Wadham C, Parker A, Wang L, Xia P. High glucose attenuates protein S-nitrosylation in endothelial cells: role of oxidative stress. *Diabetes*. 2007;56:2715–2721. doi: 10.2337/db06-1294.
289. Steffensen LB, Mortensen MB, Kjolby M, Hagensen MK, Oxvig C, Bentzon JF. Disturbed Laminar Blood Flow Vastly Augments Lipoprotein Retention in the Artery Wall: A Key Mechanism Distinguishing Susceptible From Resistant Sites. *Arterioscler Thromb Vasc Biol*. 2015;35:1928–1935. doi: 10.1161/ATVBAHA.115.305874.
290. Peiffer V, Sherwin SJ, Weinberg PD. Does low and oscillatory wall shear stress correlate spatially with early atherosclerosis? A systematic review. *Cardiovasc Res*. 2013;99:242–250. doi: 10.1093/cvr/cvt044.
291. Kwon GP, Schroeder JL, Amar MJ, Remaley AT, Balaban RS. Contribution of macromolecular structure to the retention of low-density lipoprotein at arterial branch points. *Circulation*. 2008;117:2919–2927. doi: 10.1161/CIRCULATIONAHA.107.754614.
292. von der Thüsen JH, van Berkel TJ, Biessen EA. Induction of rapid atherogenesis by perivascular carotid collar placement in apolipoprotein E-deficient and low-density lipoprotein receptor-deficient mice. *Circulation*. 2001;103:1164–1170.
293. Wight TN, Merrilees MJ. Proteoglycans in atherosclerosis and restenosis: key roles for versican. *Circ Res*. 2004;94:1158–1167. doi: 10.1161/01.RES.0000126921.29919.51.
294. Mangat R, Warnakula S, Borthwick F, Hassanali Z, Uwiera RR, Russell JC, Cheeseman CI, Vine DF, Proctor SD. Arterial retention of remnant

lipoproteins ex vivo is increased in insulin resistance because of increased arterial biglycan and production of cholesterol-rich atherogenic particles that can be improved by ezetimibe in the JCR:LA-cp rat. *J Am Heart Assoc*. 2012;1:e003434. doi: 10.1161/JAHA.112.003434.
295. Ballinger ML, Osman N, Hashimura K, de Haan JB, Jandeleit-Dahm K, Allen T, Tannock LR, Rutledge JC, Little PJ. Imatinib inhibits vascular smooth muscle proteoglycan synthesis and reduces LDL binding in vitro and aortic lipid deposition in vivo. *J Cell Mol Med*. 2010;14:1408–1418. doi: 10.1111/j.1582-4934.2009.00902.x.
296. Osman N, Getachew R, Thach L, Wang H, Su X, Zheng W, Little PJ. Platelet-derived growth factor-stimulated versican synthesis but not glycosaminoglycan elongation in vascular smooth muscle is mediated via Akt phosphorylation. *Cell Signal*. 2014;26:912–916. doi: 10.1016/j.cellsig.2014.01.019.
297. Meyers CD, Tannock LR, Wight TN, Chait A. Statin-exposed vascular smooth muscle cells secrete proteoglycans with decreased binding affinity for LDL. *J Lipid Res*. 2003;44:2152–2160. doi: 10.1194/jlr.M300252-JLR200.
298. Cardona-Sanclemente LE, Born GV. Effect of inhibition of nitric oxide synthesis on the uptake of LDL and fibrinogen by arterial walls and other organs of the rat. *Br J Pharmacol*. 1995;114:1490–1494.
299. Weinberg PD. Rate-limiting steps in the development of atherosclerosis: the response-to-influx theory. *J Vasc Res*. 2004;41:1–17. doi: 10.1159/000076124.
300. Won D, Zhu SN, Chen M, Teichert AM, Fish JE, Matouk CC, Bonert M, Ojha M, Marsden PA, Cybulsky MI. Relative reduction of endothelial nitric-oxide synthase expression and transcription in atherosclerosis-prone regions of the mouse aorta and in an in vitro model of disturbed flow. *Am J Pathol*. 2007;171:1691–1704. doi: 10.2353/ajpath.2007.060860.
301. Hahn C, Schwartz MA. Mechanotransduction in vascular physiology and atherogenesis. *Nat Rev Mol Cell Biol*. 2009;10:53–62. doi: 10.1038/nrm2596.
302. Pan S. Molecular mechanisms responsible for the atheroprotective effects of laminar shear stress. *Antioxid Redox Signal*. 2009;11:1669–1682. doi: 10.1089/ars.2009.2487.
303. Leopold JA, Loscalzo J. Oxidative risk for atherothrombotic cardiovascular disease. *Free Radic Biol Med*. 2009;47:1673–1706. doi: 10.1016/j.freeradbiomed.2009.09.009.
304. Steinberg D. The LDL modification hypothesis of atherogenesis: an update. *J Lipid Res*. 2009;50 Suppl:S376–S381. doi: 10.1194/jlr.R800087-JLR200.
305. Steinberg D, Witztum JL. Oxidized low-density lipoprotein and atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2010;30:2311–2316. doi: 10.1161/ATVBAHA.108.179697.
306. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. *Nature*. 2011;473:317–325. doi: 10.1038/nature10146.
307. Bochkov VN, Oskolkova OV, Birukov KG, Levonen AL, Binder CJ, Stöckl J. Generation and biological activities of oxidized phospholipids. *Antioxid Redox Signal*. 2010;12:1009–1059. doi: 10.1089/ars.2009.2597.
308. Binder CJ, Papac-Milicevic N, Witztum JL. Innate sensing of oxidation-specific epitopes in health and disease. *Nat Rev Immunol*. 2016;16:485–497. doi: 10.1038/nri.2016.63.
309. Cyrus T, Witztum JL, Rader DJ, Tangirala R, Fazio S, Linton MF, Funk CD. Disruption of the 12/15-lipoxygenase gene diminishes atherosclerosis in apo E-deficient mice. *J Clin Invest*. 1999;103:1597–1604. doi: 10.1172/JCI5897.
310. Cyrus T, Praticò D, Zhao L, Witztum JL, Rader DJ, Rokach J, FitzGerald GA, Funk CD. Absence of 12/15-lipoxygenase expression decreases lipid peroxidation and atherogenesis in apolipoprotein e-deficient mice. *Circulation*. 2001;103:2277–2282.
311. George J, Afek A, Shaish A, Levkovitz H, Bloom N, Cyrus T, Zhao L, Funk CD, Sigal E, Harats D. 12/15-Lipoxygenase gene disruption attenuates atherogenesis in LDL receptor-deficient mice. *Circulation*. 2001;104:1646–1650.
312. Zhao L, Cuff CA, Moss E, Wille U, Cyrus T, Klein EA, Praticò D, Rader DJ, Hunter CA, Puré E, Funk CD. Selective interleukin-12 synthesis defect in 12/15-lipoxygenase-deficient macrophages associated with reduced atherosclerosis in a mouse model of familial hypercholesterolemia. *J Biol Chem*. 2002;277:35350–35356. doi: 10.1074/jbc.M205738200.
313. Shih DM, Xia YR, Wang XP, Miller E, Castellani LW, Subbanagounder G, Cheroutre H, Faull KF, Berliner JA, Witztum JL, Lusis AJ. Combined serum paraoxonase knockout/apolipoprotein E knockout mice exhibit increased lipoprotein oxidation and atherosclerosis. *J Biol Chem*. 2000;275:17527–17535. doi: 10.1074/jbc.M910376199.
314. Hörkkö S, Bird DA, Miller E, Itabe H, Leitinger N, Subbanagounder G, Berliner JA, Friedman P, Dennis EA, Curtiss LK, Palinski W, Witztum JL. Monoclonal autoantibodies specific for oxidized phospholipids or oxidized phospholipid-protein adducts inhibit macrophage uptake of oxidized low-density lipoproteins. *J Clin Invest*. 1999;103:117–128. doi: 10.1172/JCI4533.
315. Binder CJ, Hörkkö S, Dewan A, Chang MK, Kieu EP, Goodyear CS, Shaw PX, Palinski W, Witztum JL, Silverman GJ. Pneumococcal vaccination decreases atherosclerotic lesion formation: molecular mimicry between Streptococcus pneumoniae and oxidized LDL. *Nat Med*. 2003;9:736–743. doi: 10.1038/nm876.
316. Faria-Neto JR, Chyu KY, Li X, Dimayuga PC, Ferreira C, Yano J, Cercek B, Shah PK. Passive immunization with monoclonal IgM antibodies against phosphorylcholine reduces accelerated vein graft atherosclerosis in apolipoprotein E-null mice. *Atherosclerosis*. 2006;189:83–90. doi: 10.1016/j.atherosclerosis.2005.11.033.
317. Tsiantoulas D, Diehl CJ, Witztum JL, Binder CJ. B cells and humoral immunity in atherosclerosis. *Circ Res*. 2014;114:1743–1756. doi: 10.1161/CIRCRESAHA.113.301145.
318. Kirabo A, Fontana V, de Faria AP, et al. DC isoketal-modified proteins activate T cells and promote hypertension. *J Clin Invest*. 2014;124:4642–4656. doi: 10.1172/JCI74084.
319. Pober JS. Is hypertension an autoimmune disease? *J Clin Invest*. 2014;124:4234–4236. doi: 10.1172/JCI77766.
320. Goss SP, Hogg N, Kalyanaraman B. The effect of nitric oxide release rates on the oxidation of human low density lipoprotein. *J Biol Chem*. 1997;272:21647–21653.
321. Hogg N, Kalyanaraman B, Joseph J, Struck A, Parthasarathy S. Inhibition of low-density lipoprotein oxidation by nitric oxide. Potential role in atherogenesis. *FEBS Lett*. 1993;334:170–174.
322. Kumano-Kuramochi M, Shimozu Y, Wakita C, Ohnishi-Kameyama M, Shibata T, Matsunaga S, Takano-Ishikawa Y, Watanabe J, Goto M, Xie Q, Komba S, Uchida K, Machida S. Identification of 4-hydroxy-2-nonenal-histidine adducts that serve as ligands for human lectin-like oxidized LDL receptor-1. *Biochem J*. 2012;442:171–180. doi: 10.1042/BJ20111029.
323. West XZ, Malinin NL, Merkulova AA, Tischenko M, Kerr BA, Borden EC, Podrez EA, Salomon RG, Byzova TV. Oxidative stress induces angiogenesis by activating TLR2 with novel endogenous ligands. *Nature*. 2010;467:972–976. doi: 10.1038/nature09421.
324. Mehta JL, Sanada N, Hu CP, et al. Deletion of LOX-1 reduces atherogenesis in LDLR knockout mice fed high cholesterol diet. *Circ Res*. 2007;100:1634–1642. doi: 10.1161/CIRCRESAHA.107.149724.
325. Yokoyama C, Aoyama T, Ido T, Kakino A, Shiraki T, Tanaka T, Nishigaki K, Hasegawa A, Fujita Y, Sawamura T, Minatoguchi S. Deletion of LOX-1 Protects against Heart Failure Induced by Doxorubicin. *PLoS One*. 2016;11:e0154994. doi: 10.1371/journal.pone.0154994.
326. Mullick AE, Soldau K, Kiosses WB, Bell TA 3rd, Tobias PS, Curtiss LK. Increased endothelial expression of Toll-like receptor 2 at sites of disturbed blood flow exacerbates early atherogenic events. *J Exp Med*. 2008;205:373–383. doi: 10.1084/jem.20071096.
327. Korenaga R, Ando J, Kosaki K, Isshiki M, Takada Y, Kamiya A. Negative transcriptional regulation of the VCAM-1 gene by fluid shear stress in murine endothelial cells. *Am J Physiol*. 1997;273:C1506–C1515.
328. Chappell DC, Varner SE, Nerem RM, Medford RM, Alexander RW. Oscillatory shear stress stimulates adhesion molecule expression in cultured human endothelium. *Circ Res*. 1998;82:532–539.
329. Nakashima Y, Raines EW, Plump AS, Breslow JL, Ross R. Upregulation of VCAM-1 and ICAM-1 at atherosclerosis-prone sites on the endothelium in the ApoE-deficient mouse. *Arterioscler Thromb Vasc Biol*. 1998;18:842–851.
330. Marui N, Offermann MK, Swerlick R, Kunsch C, Rosen CA, Ahmad M, Alexander RW, Medford RM. Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. *J Clin Invest*. 1993;92:1866–1874. doi: 10.1172/JCI116778.
331. Khan BV, Harrison DG, Olbrych MT, Alexander RW, Medford RM. Nitric oxide regulates vascular cell adhesion molecule 1 gene expression and redox-sensitive transcriptional events in human vascular endothelial cells. *Proc Natl Acad Sci U S A*. 1996;93:9114–9119.
332. Pueyo ME, Gonzalez W, Nicoletti A, Savoie F, Arnal JF, Michel JB. Angiotensin II stimulates endothelial vascular cell adhesion molecule-1 via nuclear factor-kappaB activation induced by intracellular oxidative stress. *Arterioscler Thromb Vasc Biol*. 2000;20:645–651.
333. Schmidt AM, Hori O, Chen JX, Li JF, Crandall J, Zhang J, Cao R, Yan SD, Brett J, Stern D. Advanced glycation endproducts interacting with

their endothelial receptor induce expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human endothelial cells and in mice. A potential mechanism for the accelerated vasculopathy of diabetes. *J Clin Invest*. 1995;96:1395–1403. doi: 10.1172/JCI118175.
334. Bouloumie A, Marumo T, Lafontan M, Busse R. Leptin induces oxidative stress in human endothelial cells. *FASEB J*. 1999;13:1231–1238.
335. Ouedraogo R, Gong Y, Berzins B, Wu X, Mahadev K, Hough K, Chan L, Goldstein BJ, Scalia R. Adiponectin deficiency increases leukocyte-endothelium interactions via upregulation of endothelial cell adhesion molecules in vivo. *J Clin Invest*. 2007;117:1718–1726. doi: 10.1172/JCI29623.
336. De Caterina R, Libby P, Peng HB, Thannickal VJ, Rajavashisth TB, Gimbrone MA Jr, Shin WS, Liao JK. Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. *J Clin Invest*. 1995;96:60–68. doi: 10.1172/JCI118074.
337. Spiecker M, Peng HB, Liao JK. Inhibition of endothelial vascular cell adhesion molecule-1 expression by nitric oxide involves the induction and nuclear translocation of IkappaBalpha. *J Biol Chem*. 1997;272:30969–30974.
338. Niu XF, Smith CW, Kubes P. Intracellular oxidative stress induced by nitric oxide synthesis inhibition increases endothelial cell adhesion to neutrophils. *Circ Res*. 1994;74:1133–1140.
339. Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc Natl Acad Sci U S A*. 1991;88:4651–4655.
340. Lehoux S. Redox signalling in vascular responses to shear and stretch. *Cardiovasc Res*. 2006;71:269–279. doi: 10.1016/j.cardiores.2006.05.008.
341. Chiu JJ, Wung BS, Shyy JY, Hsieh HJ, Wang DL. Reactive oxygen species are involved in shear stress-induced intercellular adhesion molecule-1 expression in endothelial cells. *Arterioscler Thromb Vasc Biol*. 1997;17:3570–3577.
342. Shuvaev VV, Han J, Tliba S, Arguiri E, Christofidou-Solomidou M, Ramirez SH, Dykstra H, Persidsky Y, Atochin DN, Huang PL, Muzykantov VR. Anti-inflammatory effect of targeted delivery of SOD to endothelium: mechanism, synergism with NO donors and protective effects in vitro and in vivo. *PLoS One*. 2013;8:e77002. doi: 10.1371/journal.pone.0077002.
343. Kisucka J, Chauhan AK, Patten IS, Yesilaltay A, Neumann C, Van Etten RA, Krieger M, Wagner DD. Peroxiredoxin1 prevents excessive endothelial activation and early atherosclerosis. *Circ Res*. 2008;103:598–605. doi: 10.1161/CIRCRESAHA.108.174870.
344. Sharma A, Yuen D, Huet O, Pickering R, Stefanovic N, Bernatchez P, de Haan JB. Lack of glutathione peroxidase-1 facilitates a pro-inflammatory and activated vascular endothelium. *Vascul Pharmacol*. 2016;79:32–42. doi: 10.1016/j.vph.2015.11.001.
345. Moore KJ, Sheedy FJ, Fisher EA. Macrophages in atherosclerosis: a dynamic balance. *Nat Rev Immunol*. 2013;13:709–721. doi: 10.1038/nri3520.
346. Kunjathoor VV, Febbraio M, Podrez EA, Moore KJ, Andersson L, Koehn S, Rhee JS, Silverstein R, Hoff HF, Freeman MW. Scavenger receptors class A-I/II and CD36 are the principal receptors responsible for the uptake of modified low density lipoprotein leading to lipid loading in macrophages. *J Biol Chem*. 2002;277:49982–49988. doi: 10.1074/jbc.M209649200.
347. Duewell P, Kono H, Rayner KJ, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature*. 2010;464:1357–1361. doi: 10.1038/nature08938.
348. Tabas I. Macrophage death and defective inflammation resolution in atherosclerosis. *Nat Rev Immunol*. 2010;10:36–46. doi: 10.1038/nri2675.
349. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *JAMA*. 2007;297:842–857. doi: 10.1001/jama.297.8.842.
350. Schmidt HH, Stocker R, Vollbracht C, Paulsen G, Riley D, Daiber A, Cuadrado A. Antioxidants in Translational Medicine. *Antioxid Redox Signal*. 2015;23:1130–1143. doi: 10.1089/ars.2015.6393.
351. Bast A, Haenen GR. Ten misconceptions about antioxidants. *Trends Pharmacol Sci*. 2013;34:430–436. doi: 10.1016/j.tips.2013.05.010.
352. Münzel T, Gori T, Bruno RM, Taddei S. Is oxidative stress a therapeutic target in cardiovascular disease? *Eur Heart J*. 2010;31:2741–2748. doi: 10.1093/eurheartj/ehq396.
353. Altenhöfer S, Radermacher KA, Kleikers PW, Wingler K, Schmidt HH. Evolution of NADPH Oxidase Inhibitors: Selectivity and Mechanisms for Target Engagement. *Antioxid Redox Signal*. 2015;23:406–427. doi: 10.1089/ars.2013.5814.
354. Apostolova N, Victor VM. Molecular strategies for targeting antioxidants to mitochondria: therapeutic implications. *Antioxid Redox Signal*. 2015;22:686–729. doi: 10.1089/ars.2014.5952.
355. Li H, Forstermann U. Pharmacological prevention of eNOS uncoupling. *Curr Pharm Des*. 2014;20:3595–3606.