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# Forum Review

# Endothelial Progenitor Cells, Endothelial Dysfunction, Inflammation, and Oxidative Stress in Hypertension

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## ABSTRACT

**With a prevalence in excess of 20%, hypertension is a common finding among Western adult populations. Hypertension is directly implicated in the pathophysiology of various cardiovascular disease states and is a significant contributor to ill health, leading to an excess of both morbidity and mortality. The etiology of hypertension has been explored in depth, but the pathophysiology is multifactorial, complex, and poorly understood. Recent interest has been directed toward investigating the purported role of the endothelium, which acts as an important regulator of vascular homeostasis. Endothelial dysfunction is now recognized to occur in hypertension, regardless of whether the etiology is essential or secondary to endocrine or renal processes. Nitric oxide (NO) is a volatile gas produced by endothelial cells that acts to maintain vascular tone. Reduced bioavailability of NO appears to be the key process through which endothelial dysfunction is manifested in hypertension. The result is of an imbalance of counteracting mechanisms, normally designed to maintain vascular homeostasis, leading to vasoconstriction and impaired vascular function. It has become increasingly apparent that these changes may be effected in response to enhanced oxidative stress, possibly as a result of systemic and localized inflammatory responses. This article provides an overview of endothelial dysfunction in hypertension and focuses on the purported role of oxidative stress and inflammation as the catalysts for this process.** *Antioxid. Redox Signal.* 10, 1079–1088.

## INTRODUCTION

Hypertension is a common finding among Western adult populations. The prevalence of this condition is in excess of 20%, and this figure is increasing and varies in relation to age and ethnicity (4, 52). In the 2001 Health Survey for England, the prevalence of hypertension was 3.3% in those younger than 40 years, 27.9% in those aged between 40 and 79 years, and 49.9% in those 80 years and older (52).

Of concern, hypertension is a significant contributor to ill health, resulting in an excess of both morbidity and mortality. Moreover, hypertension is directly implicated in various cardiovascular disease states, including stroke, ischemic heart disease, and peripheral vascular disease (52).

The etiology of essential hypertension has been explored in depth, but the pathophysiology is complex and multifactorial (42, 52). Recent interest has been directed toward investigating the purported role of the endothelium—the largest organ in the body—which acts as an important regulator of vascular homeostasis, maintaining a balance between vasoconstriction, vasodilatation, and regulation of smooth muscle proliferation while also providing a link with the coagulation cascade (91). This link is important, not least because endothelial dysfunction may also contribute to (or be a consequence of) various other cardiovascular processes such as atherosclerosis (13).

## ENDOTHELIAL DYSFUNCTION

A large body of evidence now exists demonstrating endothelial dysfunction in patients with hypertension (40, 68, 91). Indeed, endothelial dysfunction is recognized to occur in hy-

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pertension regardless of whether the etiology is essential or secondary to endocrine or renal processes (77). It is important to recognize that endothelial dysfunction is a functional and reversible alteration of endothelial cell function and, therein, differs significantly from endothelial damage, in which the macroscopic architecture of the endothelium is disrupted. Such endothelial damage ("destruction") may occur in association with hypertension, particularly with target-organ damage (95).

Hypertension has a direct effect on vascular function, and this process appears to occur independent of other cardiovascular risk factors. Importantly, this may relate to alterations in endothelial function (102). For example, Ward *et al*. (102) assessed 24-h ambulatory blood pressure and brachial artery endothelial and smooth muscle function in healthy volunteers and subjects with hypertension. Their results demonstrated a significant inverse correlation between systolic BP and flow-mediated dilatation (FMD). In addition, both diastolic and systolic BP had a significant inverse correlation with nitroglycerine response. These effects were largely unchanged after adjustment for body mass index, drug therapy, or other cardiovascular risk factors (102).

Further evidence for endothelial dysfunction in hypertension relates to impaired endothelium-dependent relaxation in various vascular beds (cutaneous, subcutaneous, muscle and coronary microcirculation, and peripheral and coronary macrocirculation) in response to various stimuli such as receptor-based (*e.g*., acetylcholine, bradykinin), mechanical (shear stress), or mixed (exercise and cold pressor test) (40).

Prolonged systemic hypertension results in hypertensive target-organ damage, and the most common manifestation of this is left ventricular hypertrophy (LVH). Of note, Ercan *et al*. (24) demonstrated that LVH appears to have an additional negative impact on systemic endothelial function in hypertensive patients. Other groups have also demonstrated similar findings in both the coronary and peripheral circulations of hypertensive patients (66).

Notably, the presence of endothelial dysfunction was associated with increased cardiovascular events in several studies (31, 63, 78, 86). For example, Suwaidi *et al*. (86) investigated the outcome of patients with mild coronary artery disease on the basis of endothelial function as assessed by coronary microcirculatory response to acetylcholine. They found that only patients with severe endothelial dysfunction had events during a mean follow-up of 28 months (86). Schachinger *et al*. (78) confirmed similar findings in a later study (median follow-up, 7.7 years). This group found that endothelial dysfunction (as assessed by endothelium-dependent dilator acetylcholine, sympathetic activation by cold-pressor testing, and FMD) predicted long-term atherosclerotic disease progression and cardiovascular event rates (78). In addition, endothelial dysfunction in the large peripheral circulation (FMD of brachial artery) is predictive of coronary events (63).

### *Vasoactive species*

Of the various substances produced by the endothelium, nitric oxide (NO) appears to be among the most important (15). NO is a volatile gas produced by endothelial cells that, given its lipophilic nature, is able to permeate cell membranes freely. NO rapidly induces smooth muscle relaxation leading to vasodilatation. As this process is the hallmark of endothelial dysfunction, impaired NO production or activity has been proposed as the major mechanism of endothelial dysfunction.

NO is formed by endothelial cells from its precursor L-arginine by nitric oxide synthase (NOS). This enzyme (of which three isoenzymes exist) is located on endothelial cell membranes. NOS activity is inhibited by the protein caveolin-1, which binds calmodulin. The enzyme is reactivated in response to shear stress and other vasoactive species, thereby allowing ionized calcium to bind calmodulin, thereby displacing caveolin-1 (Fig. 1) (5).

Other vasoactive species also are produced by the endothelium, some of which cause vasoconstriction. Endothelin-1 (ET-1) appears to be the principal endothelium-derived vasoconstrictor (53). ET-1 not only counteracts the effects of NO, but also promotes vascular growth. Although plasma levels of ET-1 do not seem to be increased in essential hypertension, the vasoconstrictive effect of this substance does appear to be reduced with diminished NO bioavailability (79). Thus, it is plausible that the inhibitory effect of NO on ET-1 production and activity is impaired in essential hypertension because of reduced NO availability (90). The resulting imbalance between these two vasoactive species may result in enhanced vasoconstriction (88).

## ENDOTHELIAL PROGENITOR CELLS IN HYPERTENSION

Endothelial progenitor cells (EPCs) are gaining increasing acceptance as an important marker of vascular health. These (predominantly) bone-marrow–derived stem cells are of paramount importance in the maintenance of endothelial integrity, function, and postnatal neovascularization (74). These processes are of particular importance, given that the mature endothelium has limited regenerative capacity and must rely on readily available EPCs to allow vascular repair (82). Importantly, the number and function of EPCs may reflect the balance between endothelial integrity and repair and can be used as a surrogate marker of endothelial function.

Notably, EPCs have been shown to correlate inversely with various cardiovascular risk factors (82). Of the various risk factors that alter EPC activity, hypertension has been shown to be a strong predictor of impaired EPC migratory capacity (97). Furthermore, angiotensin II appears to reduce telomerase activity within EPCs and thereby may accelerate onset of EPC senescence through increased oxidative stress (see later) (82).

In a key article, Hill *et al*. (32) demonstrated that EPCs may be reflective of cardiovascular outcome by demonstrating an association between the number of circulating EPCs and the subjects' combined Framingham risk factor score ($r$ = –0.47; $p$ = 0.001) (32). Moreover, measurement of brachial artery reactivity by FMD also revealed a significant association between endothelial function and EPC numbers (32). In another study, circulating EPCs were shown to predict the occurrence of cardiovascular events (103).

In addition, drugs such as statins, erythropoietin, and estrogens that have been shown to improve endothelial function and NO availability are potent EPC mobilizing agents (94). Indeed,

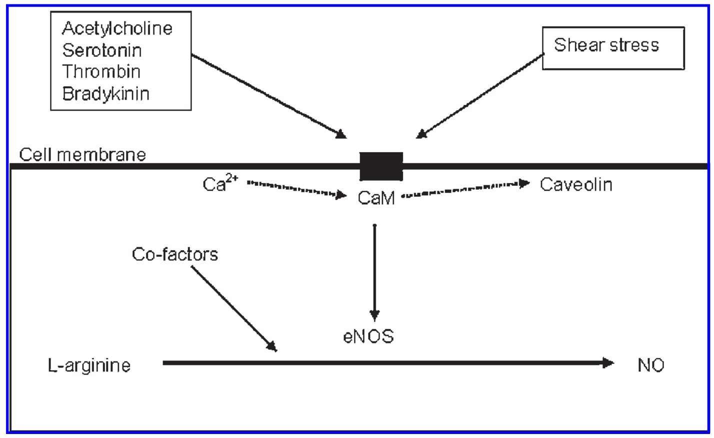


**FIG. 1. Production of nitric oxide (NO) by endothelial cells.** NO is produced from L-arginine by endothelial nitric oxide synthase (eNOS). This reaction requires several co-factors (not illustrated). Increased intracellular $Ca^{2+}$ as a consequence of shear stress or vasodilators displaces the inhibitor calveolin from calmodulin (CaM), activating eNOS. Adapted from ref. 5.

Imanishi and colleagues (38) recently extended the work of Vasa *et al*. (97) and provided further evidence for EPC dysfunction in hypertension. EPCs were cultured from rats with spontaneous hypertension and rats with hypertension induced by deoxycorticosterone acetate. Two parameters of senescence were measured: the expression of $\beta$-galactosidase and telomerase activity. In both models, EPCs derived from hypertensive rats displayed significant alterations in senescence compared with those from normal controls (38). A subsequent extension of this project investigated these findings in human subjects with and without hypertension. Once again, the researchers found reduction in telomerase activity, but also a correlation between the degree of EPC senescence and severity index of hypertension (38).

However, despite such strong initial findings, two recent studies failed to find an association between arterial hypertension and EPC numbers. Werner *et al*. (103) investigated 507 patients with coronary artery disease (432 of whom had arterial hypertension). The investigators found no association between EPC numbers and the presence of hypertension, although EPCs were predictive of cardiovascular events. More recently, Delva *et al*. (18) studied 36 patients with essential hypertension and 24 normotensive controls and found no difference in EPC number or functional activity between the groups. Of course, as acknowledged by the authors, this may simply reflect the lack of standardization of various EPC definitions.

## OXIDATIVE STRESS

Much of the available evidence for endothelial dysfunction in hypertension relates to reduced NO availability, determined by oxidative stress production, which causes enhanced NO breakdown (89).

What is oxidative stress? This commonly refers to a complicated process of cellular damage related to uncontrolled action of reactive oxygen species (ROS). These are essentially a group of molecules, including oxygen and its derivatives, produced by the normal process of aerobic metabolism. Examples of such molecules include superoxide anion ($^{\bullet}O_2^-$), hydroxyl radical ($^{\bullet}$HO), and nitric oxide ($^{\bullet}$NO). These all possess unpaired electrons and are termed "free radicals." Other ROS that have similar oxidizing ability include hydrogen peroxide ($H_2O_2$), peroxynitrite ($ONOO^-$), and hypochlorous acid (HOCl) (7). The problem arises when excess ROS exist, and normal antioxidative mechanisms are unable to cope. The resulting oxidation of biologic components (DNA, lipids, carbohydrates, protein, *etc*.) is now thought to be a major contributor to many cardiovascular diseases, including heart failure, atherosclerosis, diabetes, and hypertension, the primary effect being endothelial dysfunction.

What are the sources of oxidant stress in human physiology? As mentioned, ROS are a result of normal metabolism in cells, including those making up the endothelium. Production is potentially *via* myriad enzymatic processes, some more important contributors than others. These include xanthine oxidase, NADH/NADPH oxidases, arachidonic acid pathways, cytochrome p450s pathways, mitochondrial respiration, and eNOS. These all contribute to a reduction in endothelium-dependent vasodilation by decreasing endothelium-derived $^{\bullet}$NO. Some of the most well-known sources of ROS are discussed separately later and illustrated in Fig. 2.

### *NADH/NADPH oxidase*

Current evidence suggests that this pathway is responsible for the majority of superoxide ($^{\bullet}O_2^-$) radicals produced (48). Originally described in phagocytic immune cells (*e.g*., neutrophils), the NADH/NADPH oxidase system is now known to reside in most types of vascular cells (*i.e*., endothelial cells), smooth muscle cells, and so on (29, 67). Whereas in phagocytes, $^{\bullet}O_2^-$ is produced *via* an "oxidative burst," which can then dismutate to form another ROS, $H_2O_2$, enabling their bactericidal activity, the situation for vascular cells is probably different. For a start, the expression of the family of oxidase isoforms (NOX 1 to 5) varies between the vascular cell types, with endothelial cells expressing all the isoforms, unlike vascular smooth muscle cells, which express limited amounts. Akin to the situation in phagocytes, vascular NADPH oxidase is normally tightly regulated in health, as the products are cytotoxic. The regulatory factors include cytokines, hormones, and even mechanical forces, all known to play a role in developing cardiovascular disease (7).

When vascular smooth muscle cells are stimulated with factors such as angiotensin II, thrombin, platelet-derived growth

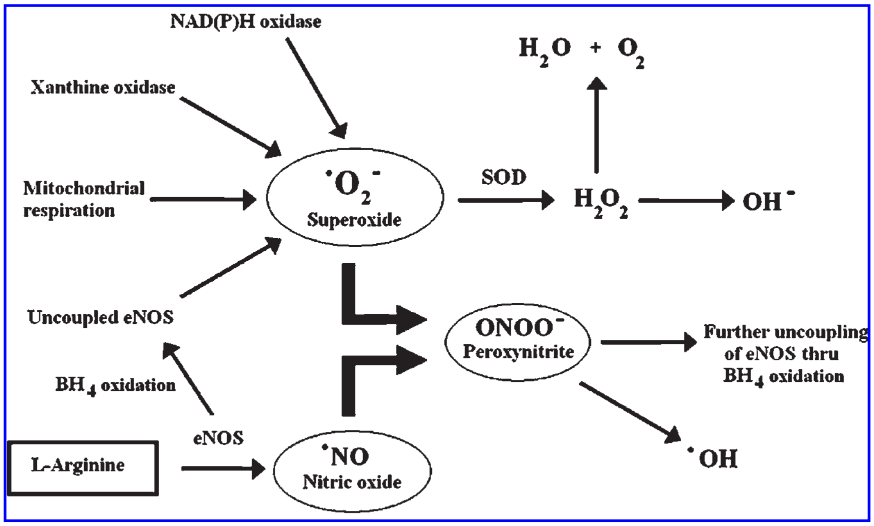


**FIG. 2. Mechanisms of oxidative stress with free radical production.**

factor, tumor growth factor, and lactosylceramide, all increase vascular ROS production and NADH/NADPH oxidase activity (6, 16, 28, 34, 54). In endothelial cells, angiotensin II stimulates an increased expression of the subunits of the enzyme (*e.g.*, NOX2) (48, 81), whereas mechanical shear stress prompts an elevation of NADH-dependent $^{\bullet}O_2^-$ formation (17). To test the relation further, it has been shown that treatments such as activators of peroxisome proliferator–activated receptor (PPAR) and HMG Co-A reductase inhibitors (statins) (known to decrease ROS levels), seem to effect a downregulation of mRNA expression responsible for the enzyme subunits (21, 55).

In cardiovascular disease, it seems that both basal and NADH-stimulated superoxide production is elevated in murine models of heart failure and myocardial infarction (3). In other models, atherosclerosis in vessels coincides with upregulation of vascular NADPH, along with a decrease in $^{\bullet}O_2^-$ formation after partial loss of the gene responsible for the enzyme (2). A reduction in the atherosclerotic lesion also is witnessed (35). All in all, there seems to be a good corresponding correlation between NADPH oxidase(s) (its subunits; activity) and ROS production in vascular cells, further suggesting that these cells produce damaging ROS *via* functional NADPH enzymes.

## *Xanthine oxidase*

Another source of ROS is through the xanthine oxidoreductase enzyme pathway. This molybdenum-conjugated enzyme is available in two forms (xanthine dehydrogenase and xanthine oxidase), which can interchange reversibly or permanently, depending on the conditions. Both forms are involved in catalyzing the conversion of xanthine to hypoxanthine and urate (purine metabolism). However, whereas xanthine dehydrogenase reduces $NAD^+$, xanthine oxidase transfers electrons to molecular oxygen, which leads to both $^{\bullet}O_2^-$ and $H_2O_2$ production and may contribute to vascular disease (81).

The location of xanthine oxidase in vascular tissue appears to be confined to endothelial cells, according to some reports (39). However, xanthine oxidase is also found in plasma, after being released from cells, and remains active with the ability to bind onto the surface of endothelial cells (81, 104). Further work has revealed that a molybdenum-deficient form of xanthine oxidase also can be found, which enables it to use NADH as an electron donor and form $^{\bullet}O_2^-$. The pathologic role for xanthine oxidase, in particular, has been suggested by studies demonstrating that it produces ROS that in turn contribute to an ischemia/reperfusion vascular injury (43, 109). In rabbits, the inhibition of the enzyme reduces $^{\bullet}O_2^-$ generation and is associated with less endothelial dysfunction (see later) (65). Xanthine oxidase is also increased in the presence of heart failure (47) and atherosclerosis (84).

## *eNOS*

This enzyme is a cytochrome p450–like reductase that catalyzes electron transport from NADPH to a heme group. Normally, this requires either L-arginine or tetrahydrobiopterin ($BH_4$) as cofactors, resulting in nitric oxide (NO) production. However, when either substrate is absent or decreased, eNOS can switch from producing $^{\bullet}$NO, to forming both $^{\bullet}O_2^-$ and $H_2O_2$ in what is now termed "eNOS uncoupling" (72). eNOS uncoupling has been confirmed *in vivo* in the setting of cardiovascular disease, including hypercholesterolemia, diabetes, smoking, nitrate tolerance, and hypertension (30, 33, 46, 50, 61). It is postulated that when eNOS uncoupling occurs in the endothelium, it affects and greatly increases oxidative stress *via* at least three mechanisms. It results in decreased $^{\bullet}$NO production as well as a concurrent increase in $^{\bullet}O_2^-$ levels, which further adds to oxidative stress. It also seems plausible that eNOS can exist in a partially uncoupled state, generating both $^{\bullet}$NO and $^{\bullet}O_2^-$. Under these conditions, the reaction between $^{\bullet}$NO and $^{\bullet}O_2^-$ results in peroxynitrite, which goes on to oxidize $BH_4$. Evidence is emerging that the action of peroxynitrite ultimately leads to further uncoupling of eNOS, thus perpetuating a dramatic surge in oxidative stress levels (7, 50).

## *Mitochondrial respiration*

Mitochondria are essential organelles present in eukaryotic cells. Mitochondrial enzymes enable the generation of ATP. However, in the course of generating a proton gradient for the production of ATP, a small percentage of electrons inevitably react with available molecular oxygen to form reactive species like $^{\bullet}O_2^-$ and $H_2O_2$. This phenomenon is potentiated by high mitochondrial membrane potentials (81) and limited by "uncoupling proteins" at the mitochondrial membrane, as well as a special variant of superoxide dismutase (SOD), which effectively eliminates $^{\bullet}O_2^-$ within the mitochondrial compartment (8). The contribution of mitochondrial ROS production to oxidant injury is particularly recognized in situations of hypoxia and diabetes (10, 64).

## ENDOTHELIAL DYSFUNCTION RELATED TO OXIDATIVE STRESS

Although strictly speaking, endothelial dysfunction encompasses all the pathologic processes involving vascular endothelium, including disordered angiogenesis and remodelling, abnormal anticoagulative conditions, inflammation, atherosclerosis, and so on, the term has become synonymous with impairment of nitric oxide ($^{\bullet}$NO)-dependent vasodilation. $^{\bullet}$NO or endothelium-derived relaxing factor (EDRF), as it was then known, is an essential component of vascular biology in health as well as disease, responsible for various actions such as vasodilation, prevention of thrombus formation, and regulating smooth muscle proliferation and leukocyte adhesiveness to vascular endothelium. When vascular $^{\bullet}$NO levels are suboptimal, this results in endothelial dysfunction. Reduced $^{\bullet}$NO may occur directly as a result of decreased or inactivated endothelial cell NO synthase (eNOS), a lack of substrate or cofactors for eNOS, and increased $^{\bullet}$NO degradation by ROS. These are all affected by and to a certain extent contribute to oxidative stress, which is proving to a primary initiator for endothelial dysfunction.

Oxidative stress produced by the likes of ROS such as $^{\bullet}O_2^-$ acts with and renders $^{\bullet}$NO inactive. Conversely, $^{\bullet}$NO is stabilized by superoxide dismutase (SOD) a natural biologic deterrent of oxidative stress, and this enables $^{\bullet}$NO to exert its vascular relaxation effect (7). Normally it is kept in equilibrium, but the stabilization and inactivation of $^{\bullet}$NO is greatly altered in the presence of cardiovascular disease or its risk factors, such as diabetes mellitus, smoking, hyperlipidemia, and hypertension, and the net effect is a profound shift toward loss of $^{\bullet}$NO, resulting in impaired vasodilation (7, 81).

Not surprisingly, these conditions are all associated with increased levels of oxidative stress. Besides a direct effect on the $^{\bullet}$NO vascular mechanism, ROS also affect low-density lipoproteins (LDLs), and the consequences include endothelial toxicity and adhesion and migration of leukocytes, first to the endothelium, followed by sequestration into the subendothelial space (81). Once oxidized, LDL also inactivates $^{\bullet}$NO and its production, thus contributing to endothelial dysfunction (11, 44).

The lack of endothelium-dependent vasodilation is now accepted as an early but reversible feature of atherosclerosis, and it is associated with a higher risk for cardiovascular adverse events and outcome (78). Some even suggest that endothelial dysfunction is responsible for the progression of stable to unstable atherosclerosis (81). The mounting evidence suggests that oxidative stress leading to the progression of endothelial dysfunction is crucial for cardiovascular disease development.

## OXIDATIVE STRESS AND HYPERTENSION

### *Evidence from Animal Studies*

In hypertension, oxidative stress has been demonstrated to be a primary contributing factor (7). In the experimental setting, studies of spontaneous hypertensive rats (SHRs) strongly suggested that ROS species (*e.g.*, $^{\bullet}O_2^-$ derived from xanthine oxidase) dramatically affected the ability of the vasculature to vasodilate. This was a result of decreased levels of bioavailable $^{\bullet}$NO. With a recombinant form of SOD (or gene transfer of SOD) to increase $^{\bullet}$NO bioavailability, blood pressure was significantly reduced in SHRs but not normotensive control rats, along with a dramatic improvement in vascular reactivity (12, 62). Similarly, administration of a xanthine oxidase inhibitor (oxypurinol) reduced blood pressure (62). Taken together with other evidence suggesting that hypertensive subjects demonstrate elevated levels of free radicals amenable to oxypurinol treatment (87), this implies a strong role for endothelial dysfunction in the pathogenesis of hypertension.

Other evidence includes the establishment of the role of NADH/NADPH oxidase in angiotensin II–induced hypertension. In hypertensive rats (through long-term administration of angiotensin II), it has been shown that NADH oxidase activity is markedly increased, with a corresponding increase specifically in vascular $^{\bullet}O_2^-$ production (75). Delivery of exogenous SOD rapidly restored vascular reactivity, with normalizing of blood pressure (49). Other studies report a stimulation of $^{\bullet}O_2^-$ production by angiotensin II in cultured vascular smooth muscle cells, resulting from increased NADH oxidase action (28). Still others report an increased mRNA expression of specific enzyme subunits for NADH oxidase in angiotensin II–induced hypertension (27).

It also is worth considering the importance of the $^{\bullet}$NO/ROS balance in the context of perinatal control, put forward by Racasan *et al*. (73). This theory proposes that a perinatal shift in $^{\bullet}$NO/ROS balance will affect adult blood pressure control. When the shift is toward increased ROS and a reduction in $^{\bullet}$NO, this results in adult hypertension. When SHRs were investigated, it was found that uncoupling of eNOS (and the resulting $^{\bullet}O_2^-$ increase) induced a "positive-feedback loop," culminating in a prolonged (or lifelong) change in the redox state. This also caused a shift in the transcription and translation of blood pressure–related control genes. By supplementing SHRs with antioxidants and L-arginine (eNOS substrate/cofactor) in the perinatal stage and in essence effecting an early "reprogramming" of redox signaling, they were able to demonstrate a reduction in mean adult blood pressure (73).

### *Evidence from human studies*

In human hypertension studies, similar evidence of a reduction in useful $^{\bullet}$NO is found, with a concomitant surge in oxidative stress levels (93). The balance of antioxidant activity (superoxide dismutase, catalase) and natural ROS scavengers (*via* vitamins E and C, glutathione, *etc*.) is upset in these hypertensive patients (26). In attempts to readdress the balance and increase antioxidant activity, it has been shown that vitamin C effectively increases $^{\bullet}$NO with subsequent improvement of endothelial function in essential hypertension (89). A possible reduction in L-arginine bioavailability may be seen: when the cofactor for eNOS is supplemented in patients with essential hypertension, a rapid improvement in endothelial function occurs, as measured by flow-mediated dilatation (FMD) studies (51).

A helpful model for understanding hypertension involves patients with renovascular disease (RVD). In the latter, activation of the renin–angiotensin system (RAS) is found, which has been implicated in the development of secondary hypertension seen in the disease. The chief mediator appears to be angiotensin II, the activity of which is greatly increased. Angiotensin II is not only a potent vasoconstrictor but has many other effects that illustrate its position as a multifunctional hormone: it enhances monocyte as well as platelet adhesion and causes proliferation of vascular smooth muscle cells and collagen (26); cellular events have been implicated in the progression of atherosclerosis and plaque genesis (25, 85). As mentioned before, prolonged infusion of angiotensin II in rats produces hypertension (75). Akin to these laboratory findings in iatrogenic hypertension, RVD hypertensive patients display an abnormal activation of RAS, which significantly correlates with increased oxidative stress, unlike in normotensive patients or essential hypertension patients (58). The findings by Minuz *et al*. (58) provide more evidence for a causative relation between renin activation (RAS) and amplified oxidative stress. It also suggests that angiotensin II is itself a stimulus for oxidative stress in RVD.

As mentioned, growing evidence suggests that the etiology of hypertension is partly a result of as well as a catalyst for further ROS production (7, 27, 45, 57, 73). It is therefore unsurprising to note that the balance of ROS and the innate cellular scavenging systems is upset in hypertensive subjects. A recent study documented higher levels of the biologic ROS scavenger superoxide dismutase (SOD) in patients compared with normotensive subjects (106). The reasons have not been fully elucidated, but presumably this is a "compensatory" biologic response to the proven high levels of ROS. This study also investigated the role of angiotensin II type 1 ($AT_1$)-receptor antagonist valsartan and demonstrated a profound effect in downregulating SOD mRNA. Interestingly, this effect was noted to occur only in hypertensive patients rather than in normotensive controls, despite similar treatment doses (106). In view of the selective results of the treatment, the study postulated that a reduction in SOD resulted from a direct reduction in ROS levels by valsartan, implying a useful antioxidant effect for valsartan. The results also imply a potential oxidative stress role for angiotensin II in essential hypertension patients, rather than just in RVD hypertension.

In hypertension, the endothelium also must contend with mechanical forces, in particular, shear stress. In general, shear stress in the laminar form is associated with eNOS and $^{\bullet}NO$ unregulation, plus increased natural antioxidant scavengers such as glutathione peroxidase and SOD (70). This encourages a healthy reactive vascular wall together with protection from oxidative stress damage. However, as in the case of hypertension, shear stress, particularly in the oscillatory aspect, leads to damaging effects through increased ROS production. In the short term, this can mean the generation of $^{\bullet}O_2^-$ and $H_2O_2$, which have been implicated in flow-induced vasodilatation in coronary and cerebral vessels (59, 69). Sustained $^{\bullet}O_2^-$ production with oscillatory shear stress, when combined with $^{\bullet}NO$ to form peroxynitrite, will further induce ROS production and exhaust normal antioxidant mechanisms.

### *Oxidative stress and endothelial progenitor cells in hypertension*

As mentioned, EPCs are now thought to be crucial for vascular health and are increasingly shown to be useful surrogate markers for cardiovascular disease and its risk factors, including hypertension (97). As oxidative stress is also associated with cardiovascular disease and risk, it is entirely plausible to investigate a link between levels and function of EPCs in the presence of oxidative stress.

A recent study showed that angiotensin II stimulates EPCs to express gp91$^{phox}$ (a major NADH oxidase subunit) (101). The findings also found that levels of peroxynitrite were elevated as a result of angiotensin II, with a corresponding increase in EPC senescence *via* telomerase inactivation. Taken together, this implied excess oxidative stress affecting EPC function and proliferative ability.

ROS also contribute to LDL oxidation: once oxidized, LDL plays a role in endothelial dysfunction by inactivating $^{\bullet}NO$ and its production (29, 48). Besides their effects on $^{\bullet}NO$, oxidized LDL (oxLDL) is now known to affect EPCs as well. First, oxLDL appears to reduce the number and function of EPCs in a concentration-dependent manner (36). Furthermore, the capacity of EPCs to proliferate, migrate, and adhere to fibronectin is drastically impaired. The cause for the functional change in EPC ability, as measured by *in vitro* studies, appears to stem from oxLDL-induced senescence of EPCs (37) by diminishing telomerase activity. By adding either specific oxLDL-receptor antibody or a statin (atorvastatin), the same study was able to demonstrate a significant reduction in telomerase inhibition, thereby providing a novel mechanism by which statins reduce oxidative stress effects on EPCs. It has been shown that HMG-CoA reductase inhibitors like atorvastatin effectively increased functional circulating EPCs in patients with stable cardiovascular disease (96).

## INFLAMMATION, OXIDATIVE STRESS, AND ENDOTHELIAL DYSFUNCTION

The potential role for low-grade systemic inflammation to drive various disease states has become increasingly appreciated. The most studied marker of inflammation is high-sensitivity C-reactive protein (hsCRP). This circulating acute-phase reactant is synthesized in the liver primarily in response to interleukin-6 (IL-6) and IL-1$\beta$ and shows remarkable potential as a predictor of future cardiovascular events in those with preexisting cardiovascular disease (19, 105). Even in apparently healthy subjects, hsCRP has emerged as a strong, reproducible, and independent risk factor for future cardiovascular events (14).

Crucially, hsCRP has been related to systolic BP, pulse pressure, and incident hypertension (9, 23) and also to markers of endothelial dysfunction (*e.g.*, von Willebrand factor, tissue plasminogen activator) (108). Curiously, no correlation was found between hsCRP and diastolic BP (1, 80). Other groups have related hsCRP levels to increased large-artery stiffness and reduced elasticity, potentially also contributing to increased blood pressure (22, 56, 107). Thus, CRP may be more than purely a marker of increased cardiovascular risk, but may be intimately involved in the promotion of endothelial dysfunction.

In endothelial cells, CRP has been shown to facilitate release of ET-1 (60, 98), reduce NO synthase (83) and the bioavailability of NO (71), and consequently reduce NO-mediated vasodilatation. Furthermore, in vascular smooth muscle cells, CRP induced expression of angiotensin-1 (AT-1) receptors, thereby enhancing ROS formation, further reducing NO bioavailability (99). CRP also displays the ability to reduce prostacyclin formation (another) and reduces EPC survival, differentiation, and function (92).

### *Antihypertensive therapy*

As already discussed, given the intimate relation between the renin–angiotensin–aldosterone system and NO formation, it would be expected that drugs modulating this pathway may display beneficial effects on endothelial function. Losartan has been shown to attenuate CRP-induced upregulation of the AT-1 receptor. However, neither losartan, irbesartan, nor candesartan displays any impact in reducing serum CRP in hypertensive patients (100). In the recent Val-MARC (Valsartan Managing blood pressure Aggressively and evaluating Reductions in hsCRP) study, valsartan may have a minor impact on hsCRP levels (76). Incidentally, this effect was lost when valsartan was combined with a thiazide diuretic (76).

## CONCLUSIONS

Hypertension is an important cardiovascular risk factor and contributes to growing ill health. Numerous drugs are available, and the significant benefits of optimizing blood pressure control are clear. Nevertheless, it is worrying that despite the success of such therapies, our knowledge and understanding of the basic pathophysiologic and causative processes remain limited, given the fact that the pathophysiology of this condition is multifactorial, complex, and poorly understood. Recent interest has been directed toward investigating the purported role of the endothelium, which acts as an important regulator of vascular homeostasis. Endothelial dysfunction is now clearly recognized to be central to the pathophysiology of hypertension, regardless of whether the etiology is essential or secondary to endocrine or renal processes.

## ABBREVIATIONS

$AT_1$, Angiotensin II type 1; ATP, adenosine triphosphate; BP, blood pressure; EDRF, endothelium-derived relaxing factor; eNOS, endothelial nitric oxide synthase; EPC, endothelial progenitor cell; ET-1, endothelin-1; FMD, flow-mediated dilatation; $H_2O_2$, hydrogen peroxide; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl co-enzyme A reductase; HO, hydroxyl radical; HOCl, hypocholorous acid; hsCRP, high-sensitivity C-reactive protein; IL-1$\beta$, interleukin-1$\beta$; IL-6, interleukin-6; LDL, low-density lipoprotein; LVH, left ventricular hypertrophy; mRNA, messenger ribonucleic acid; NAD, nicotinamide adenine dinucleotide; NADH, reduced/hydrogenated nicotineamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; NOS, nitric oxide synthase; NOX, oxides of nitrogen; $O_2^-$, superoxide anion; $ONOO^-$, peroxynitrite; oxLDL, oxidized low-density lipoprotein; PPAR, peroxisome proliferator-activated receptor; RAS, renin–angiotensin system; ROS, reactive oxygen species; RVD, renovascular disease; SHR, spontaneous hypertensive rat; SOD, serum oxide dismutase; ValMARC, Valsartan Managing blood pressure Aggressively and evaluating Reductions in hsCRP clinical trial;

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