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# Forum Review

# Nitric Oxide, Tetrahydrobiopterin, Oxidative Stress, and Endothelial Dysfunction in Hypertension

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## ABSTRACT

**Endothelial dysfunction in the setting of cardiovascular risk factors such as hypercholesterolemia, diabetes mellitus, chronic smoking, as well hypertension, is, at least in part, dependent of the production of reactive oxygen species (ROS) and the subsequent decrease in vascular bioavailability of nitric oxide (NO). ROS-producing enzymes involved in increased oxidative stress within vascular tissue include NADPH oxidase, xanthine oxidase, and mitochondrial superoxide producing enzymes. Superoxide produced by the NADPH oxidase may react with NO, thereby stimulating the production of the NO/superoxide reaction product peroxynitrite. Peroxynitrite in turn has been shown to uncouple eNOS, therefore switching an antiatherosclerotic NO producing enzyme to an enzyme that may accelerate the atherosclerotic process by producing superoxide. Increased oxidative stress in the vasculature, however, is not restricted to the endothelium and also occurs within the smooth muscle cell layer. Increased superoxide production has important consequences with respect to signaling by the soluble guanylate cyclase and the cGMP-dependent kinase I, which activity and expression is regulated in a redox-sensitive fashion. The present review will summarize current concepts concerning eNOS uncoupling, with special focus on the role of tetrahydrobiopterin in mediating eNOS uncoupling.** *Antioxid. Redox Signal.* 10, 1115–1126.

## INTRODUCTION

TRADITIONALLY, THE ROLE OF THE ENDOTHELIUM was thought primarily to be that of a selective barrier to the diffusion of macromolecules from the blood lumen to the interstitial space. During the past 20 years, numerous additional roles for the endothelium have been defined, such as regulation of vascular tone, modulation of inflammation, promotion as well as inhibition of vascular growth, and modulation of platelet aggregation and coagulation. Endothelial dysfunction is a characteristic feature of patients with coronary atherosclerosis, and more recent studies indicate that it may predict long-term atherosclerotic disease progression as well as cardiovascular event rate (77). Although the mechanisms underlying endothelial dysfunction are multifactorial, there is a growing body of evidence that increased reactive oxygen species (ROS) production may contribute considerably to this phenomenon. ROS production has been demonstrated to occur in the endothelial cell layer, but also within the media and adventitia, all of which may impair NO signaling within vascular tissue to endothelium-dependent, but also endothelium-independent vasodilators. More recent experimental, but also clinical studies point to a crucial role of eNOS as a ROS producing enzyme in the setting of arterial hypertension. This review will briefly address mechanisms underlying eNOS uncoupling and will focus primarily on the consequences of intracellular absolute or relative deficiency of the eNOS cofactor tetrahydrobiopterin in causing eNOS uncoupling.

## OXIDATIVE STRESS CAUSES ENDOTHELIAL DYSFUNCTION

The endothelium-derived relaxing factor, previously identified as nitric oxide (NO) (56) or a closely related compound

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(50), has potent antiatherosclerotic properties. Nitric oxide released from endothelial cells works in concert with prostacyclin to inhibit platelet aggregation (62), it inhibits the attachment of neutrophils to endothelial cells, and the expression of adhesion molecules. Nitric oxide in high concentrations inhibits the proliferation of smooth muscle cells (16). Therefore, under all conditions where an absolute or relative nitric oxide deficit is encountered, the process of atherosclerosis is being initiated or accelerated. The half-life of nitric oxide and therefore its biological activity is decisively determined by oxygen-derived free radicals such as superoxide (21). Superoxide rapidly reacts with nitric oxide to form the highly reactive intermediate peroxynitrite (4). The rapid bimolecular reaction between nitric oxide and superoxide yielding peroxynitrite ($ONOO^-$, rate constant: $5–10 \times 10^9\ M^{-1}s^{-1}$) is ~3–4 times faster than the dismutation of superoxide by the superoxide dismutase. Therefore, peroxynitrite formation represents a major potential pathway of nitric oxide reactivity, pending on the rates of tissue superoxide production. Peroxynitrite in high concentrations is cytotoxic and may cause oxidative damage to proteins, lipids, and DNA (3). Recent studies also indicate that $ONOO^-$ may have deleterious effects on activity and function of prostacyclin synthase (92) and the endothelial NOS (91). Other reactive oxygen species (ROS) such as the dismutation product of superoxide, hydrogen peroxide, and hypochlorous acid cannot be considered as free radicals, but have a powerful oxidizing capacity, which will further contribute to oxidative stress within vascular tissue.

## ENDOTHELIAL DYSFUNCTION AND CARDIOVASCULAR RISK FACTORS

It is well known that, in the presence of cardiovascular risk factors, endothelial dysfunction is frequently encountered. This has been shown for chronic smokers, patients with increased LDL levels, for patients with diabetes Types I and II, for hypertensive patients and for patients with metabolic syndrome. There are several potential abnormalities, which could account for reductions in endothelium-dependent vascular relaxation, including changes in the activity and/or expression of the eNOS, decreased sensitivity of vascular smooth muscle cells to NO, or increased degradation of NO via its interaction with ROS such as superoxide. The NO-degradation concept is the most attractive one since in the presence of cardiovascular risk factors such as diabetes mellitus, hypertension, chronic smoking, a positive family history for the development of coronary artery disease, as well as hyperlipidemia, endothelial dysfunction is established and even more importantly it is markedly improved by the acute administration of the antioxidant vitamin C (13, 25, 41, 80).

## ENDOTHELIAL DYSFUNCTION AND PROGNOSIS

Since 2000, a quite large number of clinical trials have demonstrated a quite close association between coronary and peripheral endothelial function and the likelihood of cardiovascular events. For example Volker Schächinger from Andreas Zeiher's group has shown that patients who respond to intracoronary acetylcholine (ACh) with vasodilation have subsequently much less cardiovascular events defined as a composite endpoint including cardiovascular death, unstable angina, myocardial infarction, coronary revascularization, ischemic stroke, and peripheral artery revascularization (66), as compared to patients who responded to intracoronary ACh with vasoconstriction. More recently, we were able to show that peripheral endothelial function also has prognostic meanings (27). Patients with cardiovascular events had a clearly attenuated response to intrabrachial infusion in forearm blood flow established with the forearm plethysmography method. Even more exciting, we could demonstrate that patients who responded well to intraarterial infusion with vitamin C with an improvement of endothelial function had a worse prognosis as compared to patients with a low or no vitamin C effects (27) (Fig. 1). This finding not only strengthens the concept that oxidative stress indeed is the key player in determining the degree of endothelial dysfunction but also the prognosis in patients with established coronary artery disease. Further studies revealed a clear-cut association between endothelial dysfunction and prognosis in patients with chronic congestive heart failure (24), essential hypertension (57), and peripheral artery disease (18). For example, in an exciting study, Gokce *et al*. quantified endothelial function in patients undergoing peripheral or coronary bypass surgery (17). These patients have a high perioperative event rate within the first 30 days after surgery. In all patients, flow mediated dilation (FMD) of the brachial artery was measured before surgery and the patients were grouped into three tertiles; FMD $> 8\%$ between 4%–8% and below 4%. The authors found that patients with a FMD $> 8\%$ had almost no cardiovascular events during a follow-up period of 30 days, while patients with an FMD $< 8\%$ had substantially more events (17). This study actually implies that measurement of endothelial function in the brachial artery may provide information about plaque stability in coronary arteries.

In patients with essential hypertension, Perticone *et al*. showed that in 225 never-treated patients with essential hypertension, that those patients with peripheral endothelial dysfunction in forearm arterioles had clearly more cardiovascular event rates as compared to patients with good endothelial function (57). The excess risk in patients with the worst endothelial function was still significant after controlling for individual risk markers (Fig. 2A). The same group also showed that the co-existence of left ventricular hypertrophy and endothelial dysfunction in hypertensive patients increases significantly the risk of subsequent cardiovascular events (69) (Fig. 2B).

Taken together, there is no doubt that measurement of endothelial function provides substantial prognostic information about future cardiovascular events in secondary prevention, whereas its role in primary prevention remains to be established.

## MECHANISMS UNDERLYING INCREASED OXIDATIVE STRESS

### *eNOS uncoupling contributes to endothelial dysfunction*

In most situations where endothelial dysfunction due to increased oxidative stress is encountered, the expression of the eNOS has been shown to be paradoxically increased rather than decreased (22, 29, 40, 83). The mechanisms underlying increased expression of eNOS is likely to be secondary to in-

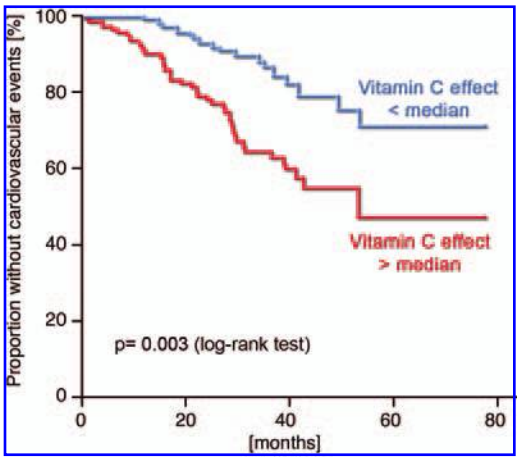


**FIG. 1. Kaplan–Meier analysis demonstrating cumulative proportion of patients without cardiovascular events during follow up.** Effects of vitamin C on ACh-induced forearm dilation is divided into values below and above the median. The data clearly show that patients with a strong vitamin C response, reflecting high oxidative stress in the vasculature, have subsequently more cardiovascular events, compared to patients with a weaker vitamin C response (adapted from ref. 27). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

creased endothelial levels of hydrogen peroxide, which increases the expression of eNOS at the transcriptional and translational level (12). The demonstration of endothelial dysfunction in the presence of increased expression of eNOS indicates that the capacity of the enzyme to produce NO may be limited. Very intriguing are observations that the eNOS itself can be a superoxide source, thereby causing endothelial dysfunction (14).

### *Studies with the isolated enzyme*

It has become clear from studies with the purified enzyme that eNOS may become "uncoupled" [*e.g.*, in the absence of the NOS substrate L-arginine or the cofactor tetrahydrobiopterin ($BH_4$)]. In such uncoupled state, electrons normally flowing from the reductase domain of one subunit to the oxygenase domain of the other subunit are diverted to molecular oxygen rather than to L-arginine (81, 89), resulting in production of superoxide rather than nitric oxide (Fig. 3). There are several possibilities how eNOS uncoupling may occur.

### *eNOS uncoupling due to increased peroxynitrite-mediated $BH_4$ oxidation*

Tetrahydrobiopterin seems to be essential for the time-critical delivery of one electron to an intermediate in the catalytic cycle of NOS. The resulting species releases NO and L-citrulline. The $BH_3$ radical is reduced by the iron, completing the cycle by formation of $Fe^{III}$ and $BH_4$. In the absence of $BH_4$, the intermediate reacts with molecular oxygen resulting in superoxide formation.

The first evidence that eNOS uncoupling may be a pathophysiologically relevant phenomenon was provided by *in vitro* studies where it was demonstrated that native LDL (61), and even more pronounced oxidized LDL (84), are able to stimulate endothelial superoxide production and that this phenomenon is inhibited by the NOS inhibitor L-NAME, pointing to a specific role of eNOS in superoxide production. These *in vitro* observations were rapidly extended by *in vivo* studies in animals with hypercholesterolemia (52), diabetes mellitus (29), angiotensin II hypertension (47), and chronic congestive heart failure (46), where an uncoupled eNOS was consistently identified as a significant superoxide source. In the next paragraphs, we

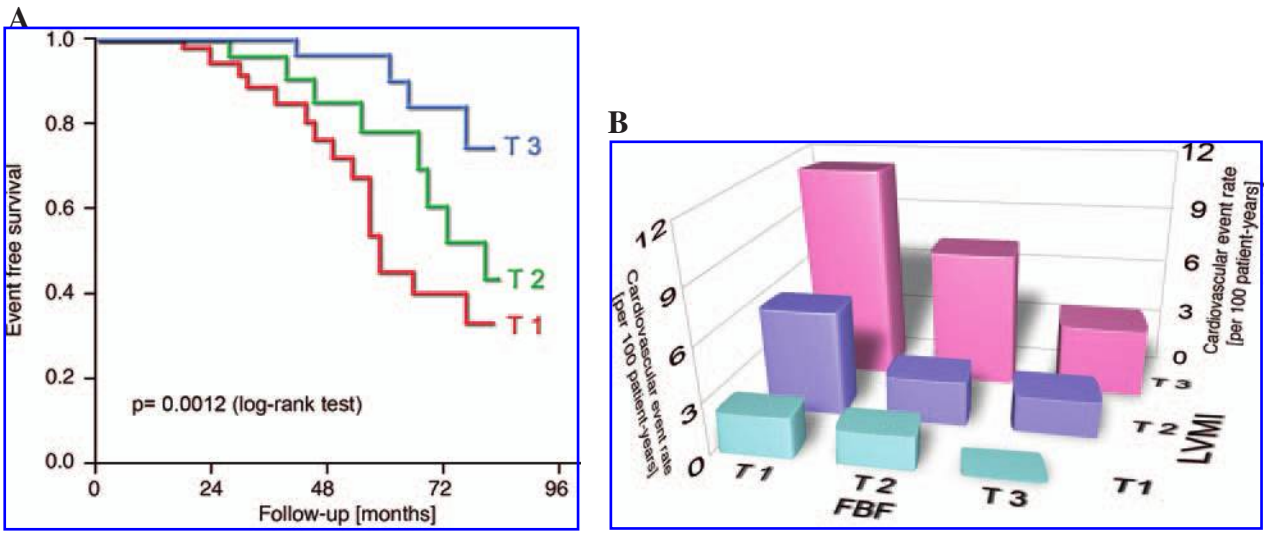


**FIG. 2. (A) Kaplan–Meier Analysis.** Event free survival curves in patients with essential hypertension were subdivided into tertiles of forearm blood flow (FBF). Endothelium-dependent increases in forearm blood flow were achieved with intrabrachial infusion of the endothelium dependent vasodilator acetylcholine. The cumulative cardiovascular events rate in the first tertile was 52% in 7 years, compared with a cumulative event rate of 14% in the third tertile (adapted from ref. 57). (**B**) Rate of total cardiovascular events in relation to tertiles of echocardiographic mass and peak percentage increase in acetylcholine-induced vasodilation. The figure clearly shows that the rate of total cardiovascular events significantly increases from the first to the third tertile of left ventricular mass index (LVMI) (adapted from ref. 69). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

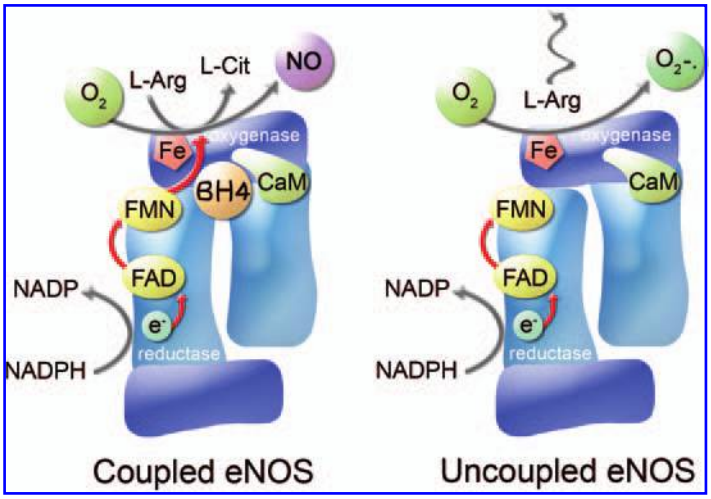


**FIG. 3. Electron flow in coupled *versus* uncoupled eNOS.** Electron flow starts from NADPH to flavins FAD and FMN in the reductase domain, which delivers the electrons to the iron of the heme (oxygenase domain) and to the $BH_3$, radical generated as an intermediate in the catalytic cycle. $BH_4$ seems to be essential to donate an electron and proton to versatile intermediates in the reaction cycle of L-arginine/$O_2$ to citrullin/NO. Calmodulin (CAM) controls electron flow in eNOS. When $BH_4$ is limiting, electron transfer becomes uncoupled to L-arginine oxidation, the ferrous dioxygen complex dissociates, and superoxide ($O_2^{-}$.) is generated from the oxygenase domain (adapted from ref. 68). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

will discuss the potential mechanisms leading to eNOS uncoupling in vascular disease.

## *Decreased $BH_4$ levels and decreased expression of the CTP cyclohydrolase I*

Strategies to improve endothelial function (*e.g.*, in atherosclerosis) via increasing the expression of eNOS have yielded mixed results. Ozaki and coworkers reported that targeted overexpression of eNOS in ApoE knockout animals accelerated rather than decreased atherosclerotic lesion formation (55). The authors also established a marked decrease in vascular $BH_4$ content and identified the endothelium as the pivotal superoxide source compatible with eNOS uncoupling. Treatment with $BH_4$ reduced lesion formation, reduced vascular superoxide, and increased endothelial NO production (55). These findings clearly indicate that the stimulation of increases in eNOS expression without simultaneously increasing vascular levels of $BH_4$ may lead to eNOS uncoupling due to the stoichiometric relationships between endothelial $BH_4$ and NOS activity (5).

One very attractive concept to explain intracellular $BH_4$ depletion is the oxidative modification of $BH_4$ (38). In the setting of an activation of a vascular superoxide source, superoxide will react with NO to form the highly reactive intermediate peroxynitrite. Peroxynitrite in turn will oxidize $BH_4$ to the $BH_3^{\cdot}$ radical, and the resulting decrease in endothelial $BH_4$ triggers eNOS uncoupling (Fig. 3).

This concept, however, also implies that the uncoupling of eNOS is mediated by $ONOO^{-}$ and would invariably require a priming event such as superoxide produced by NADPH oxidase, xanthine oxidase, or mitochondria. These so-called "kindling radicals" would lead to eNOS uncoupling via increased formation of peroxynitrite and subsequent eNOS-mediated superoxide production (bonfire radical).

Oxidation of $BH_4$ not only reduces $BH_4$ bioavailability, but the oxidation products such as $BH_2$ may compete with $BH_4$ for binding to eNOS (82), thereby leading to eNOS uncoupling. Interestingly, vitamin C was able to recycle the $BH_3^{\cdot}$ radical to $BH_4$ but not $BH_2$ to $BH_4$. This observation may indicate that the beneficial effects of vitamin C on endothelial function in patients may be explained in part by a recycling of the $BH_3^{\cdot}$ radical to $BH_4$, rather than by directly scavenging superoxide (38).

In addition to peroxynitrite-mediated oxidation of $BH_4$, there may be also an intracellular decrease due to decreased synthesis by inhibition of the GTP cyclohydrolase I (GTP-CH 1; *de novo* synthetic pathway; Fig. 4) or by inhibition of the so-called salvage pathway involving enzymes such as sepiapterin synthase, sepiapterin reductase, or dihydrofolate reductase (DHFR; Fig. 4). Interestingly, recent studies indicate that, for example, in the setting of angiotensin II-induced hypertension, a downregulation of the DHFR was observed that was accompanied by decreased vascular $BH_4$ levels and an uncoupled eNOS (7).

In the case of eNOS uncoupling due to relative intracellular $BH_4$ deficiency, this phenomenon could be prevented if simultaneously the expression of the $BH_4$ synthesizing enzyme, GTP-CH I, were upregulated. This has been demonstrated in isolated cells and in atherosclerotic animal models. More recent clinical data also indicate that variants of the GTP-CH I are governing nitric oxide production, autonomic activity, and in addition, cardiovascular risk (90).

Another proof of the concept that $BH_4$-mediated eNOS uncoupling contributes to endothelial dysfunction was delivered by experiments demonstrating that supplementation with $BH_4$ and by the $BH_4$ precursor sepiapterin was able to improve endothelial dysfunction. $BH_4$ has been shown to improve endothelial dysfunction in smokers (26), diabetic subjects (58), hypertensive patients (28), patients with hypercholesterolemia (75), and those with coronary artery disease (70). It is important to note that $BH_4$ per se has antioxidant properties. Therefore, it may be difficult to differentiate whether the $BH_4$-induced improvements in endothelial dysfunction are due to recoupling of eNOS or due to the antioxidant properties of $BH_4$ itself. To differentiate these nonspecific *versus* specific effects on endothelial dysfunction, one has to use the pteridine analogue tetrahydroneopterin ($NH_4$), which has comparable antioxidant properties, but no effect at all on an uncoupled eNOS (26) (Fig. 5).

Endothelial dysfunction in the setting of coronary artery disease was also improved by treatment with folic acid (86). Recent experimental studies revealed that folate triggers these beneficial effects by an enhancement of binding affinity of $BH_4$ to NOS by a pteridine-binding domain serving as a locus through which the

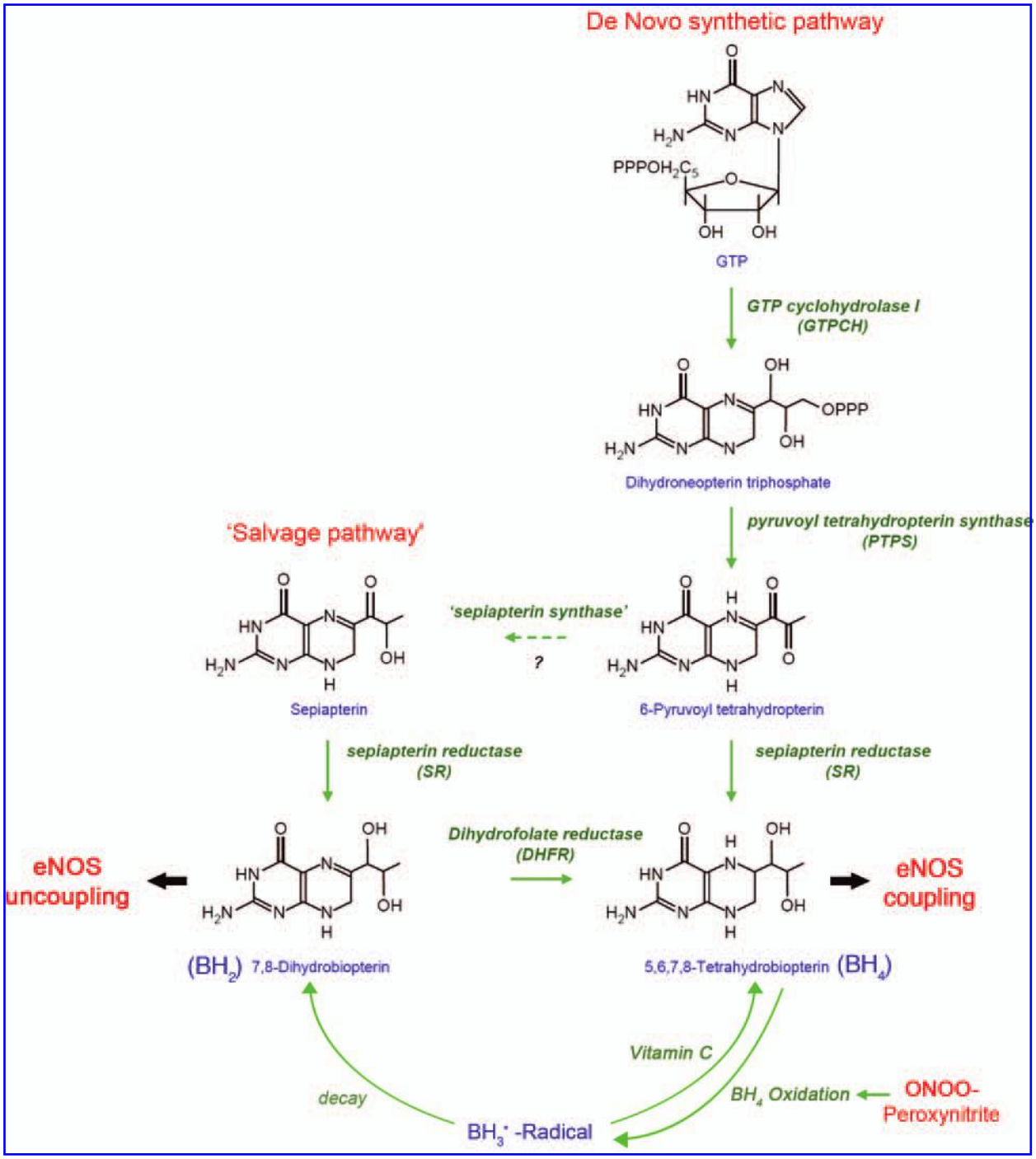


**FIG. 4. Pathways of $BH_4$ synthesis and degradation.** $BH_4$ biosynthesis proceeds from GTP via 7,8-dyhydroneopterin triphosphate and 6-pyruvoyl-5,6,7,8-tetrahydropterin. The first and rate-limiting step in the pathway is GTP cyclohydrolase (GTP-CH). Subsequent steps are catalyzed by the enzymes 6-pyruvoyl tetrahydropterin synthase and sepiapterin reductase. An alternative pathway for $BH_4$ synthesis whereby 6-pyruvoyl-5,6,7,8-tetrahydropterin is converted to sepiapterin by an enzyme termed "sepiapterin synthase." There is limited evidence for a sepiapterin synthesis pathway in mammals. However, exogenous sepiapterin can be reduced in all cells by sepiapterin reductase to $BH_2$, and further by dihydrofolate reductase to form $BH_4$ (the so-called salvage pathway). $ONOO^-$ oxidizes $BH_4$ to the intermediate $BH_3$. radical, which can decay to $BH_2$ or can be converted back to $BH_4$ by ascorbate (38). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

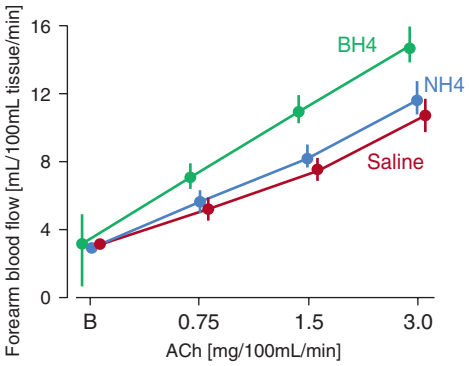


**FIG. 5. Effect of tetrahydrobiopterin ($BH_4$) and tetrahydroneopterin ($NH_4$) on the ACh dose-response relationship in chronic smokers.** $BH_4$ significantly improved ACh dose-response relationship, whereas $NH_4$ was ineffective. Adapted from ref. 26. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

active form 5-methyl tetrahydrofolate (5-MTHF) facilitates the electron transfer by $BH_4$ from the NOS reductase domain to heme (76). Folate also enhances regeneration of $BH_4$ from inactive $BH_2$ by stimulating DHFR and it chemically stabilizes $BH_4$ (85).

### *eNOS uncoupling due to peroxynitrite-mediated oxidation of the zinc–thiolate complex*

Another interesting concept concerning eNOS uncoupling was provided by Zou *et al*. (91). The authors showed that the exposure of the isolated enzyme to the oxidant peroxynitrite leads to a disruption of the zinc–thiolate cluster, resulting in an uncoupling of the enzyme (91). The authors also demonstrated that a similar phenomenon occurs when endothelial cells were exposed to high concentrations of glucose. Additional experiments revealed that $BH_4$ was oxidized at concentrations being 10- to 100-fold higher than those needed to disrupt the zinc–thiolate complex. Based on the findings, the authors suggested that the principal mechanism of uncoupling is rather the oxidation of the zinc–thiolate center and the subsequent release of zinc rather than the $BH_4$ oxidation process (91).

### *Potential role of L-arginine in eNOS uncoupling*

Beneficial effects of L-arginine supplementation have been documented in both animal studies and humans under pathophysiological conditions such as hypercholesterolemia and hypertension (11, 30, 32, 64). However, based on *in vitro* studies, it appears unlikely that L-arginine concentrations will ever become critical as a substrate *in vivo*: the $K_M$ of eNOS for L-arginine is ~3 $\mu$mol/L (59), L-arginine plasma concentrations are ~100 $\mu$mol/L, and there is a ~10-fold accumulation of L-arginine within cells (9). Thus, nonsubstrate effects of L-arginine are likely to explain the beneficial effects quoted above. These include potential direct radical scavenging properties of the guanidino nitrogen group, the cooperativity between L-arginine and $BH_4$ binding sites of NOS (19, 45), or the competition of L-arginine with the derivative asymmetric dimethyl-L-arginine (ADMA), which is an endogenous inhibitor of eNOS activity (see below) (78).

### *eNOS uncoupling and increased production of asymmetric dimethyl arginine (ADMA)*

Several recently published studies demonstrate that increased concentrations of ADMA in cultured endothelial cells or in patients with endothelial dysfunction are associated with increased ROS production (6, 42, 79). The question is whether increased ROS production is the reason for increased ADMA levels or whether increased production of ADMA actually contributes to the oxidative stress burden of the vasculature via uncoupling of eNOS. Interestingly, the activity of methylating enzymes such as the *S*-adenosylmethionine-dependent protein arginine methyltransferase (PRMT; Type I) (6) is responsible for the ADMA synthesis or the activity of ADMA hydrolyzing enzymes such as dimethylarginine dimethylaminohydrolase (DDAH) (42) is redox-sensitive. Thus, oxidative stress in the vasculature should always stimulate ADMA production and/or inhibit ADMA degradation, in concentrations that significantly inhibit eNOS activity or even uncouple the enzyme which would further increase superoxide production in a positive feedback fashion (78).

## DOES NADPH OXIDASE PRODUCE THE KINDLING RADICAL FOR INCREASED PEROXYNITRITE FORMATION ULTIMATELY LEADING TO ENOS UNCOUPLING?

The NADPH-oxidase is a superoxide producing enzyme that was first characterized in neutrophils (1). Meanwhile, we know that a similar enzyme exists also in endothelial and smooth muscle cells, as well as in the adventitia. The activity of the enzyme in endothelial and smooth muscle cells is increased upon stimulation with angiotensin II (20). The stimulatory effects of angiotensin II on the activity of this enzyme would suggest that in the presence of an activated renin angiotensin system (local or circulating), vascular dysfunction due to increased vascular superoxide production is likely to be expected. Experimental hypercholesterolemia has been shown to be associated with an activation of NADPH oxidase (87); and there is a close association between endothelial dysfunction and clinical risk factors on one hand and the activity of this enzyme in human saphenous veins in patients with coronary artery disease on the other hand (23). In atherosclerotic arteries, there is evidence for increased expression of the NADPH oxidase subunit gp91phox and nox-4, all of which may contribute to increased oxidative stress within vascular tissue (74).

Interestingly, there is a growing body of evidence that the local renin angiotensin system is activated in hypercholesterolemia. In patients, ACE-activity and therefore local angiotensin II concentrations are increased in atherosclerotic plaques (10, 53) and inflammatory cells are capable of producing large amounts of angiotensin II. Increased angiotensin II concentrations along with increased levels of superoxide have been shown in the shoulder region of atherosclerotic plaques (67). In vessels from hypercholesterolemic animals (87), as well as in platelets from hypercholesterolemic patients (51), there is an increase in the expression of the angiotensin II receptor sub-

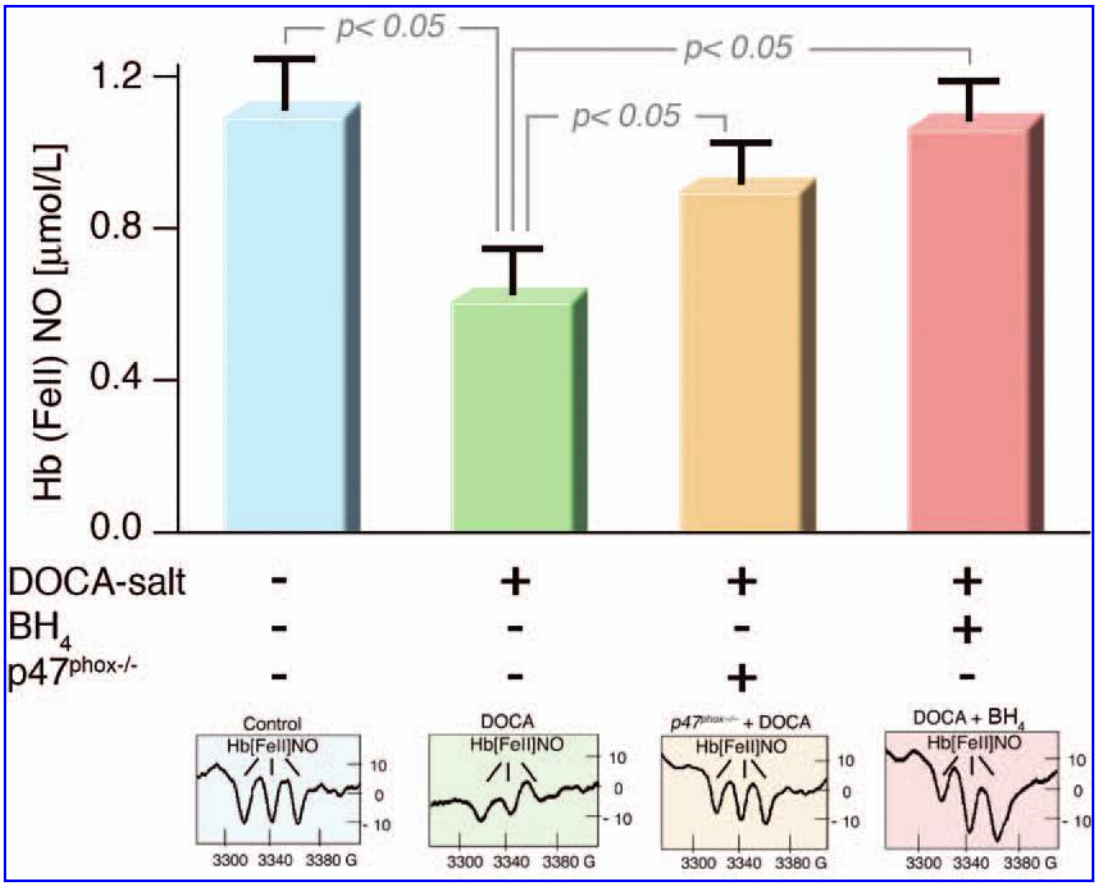


**FIG. 6. Effects of NADPH oxidase deficiency ($p47^{phox-/-}$) and tetrahydrobiopterin ($BH_4$) treatment on NO production as detect by paramagnetic resonance.** In animals with DOCA-salt hypertension, vascular NO production was clearly diminished, compatible with eNOS uncoupling. Knockout of an NADPH oxidase subunit as well as tetrahydrobiopterin ($BH_4$) treatment normalized or clearly improved vascular NO production, strongly suggesting that an activation of the vascular NADPH oxidase in this hypertension model represents the "priming event" leading to eNOS uncoupling (adapted from ref. 39). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

type $AT_1$. Thus, both experimental and clinical studies have provided evidence for stimulation of the renin angiotensin system in atherosclerosis and simultaneously for an activation of NADPH oxidase in the arterial wall. Similar evidence for an activation of this enzyme in the vasculature has been provided from experimental animal models of different forms of hypertension such as angiotensin II infusion (15, 63) and in spontaneously hypertensive rats (SHR) (48), as well as in different forms of diabetes mellitus (29). In animal models of angiotensin II-induced hypertension (47), streptozotocin- induced diabetes, as well as in patients with diabetes mellitus, increased ROS production by NADPH oxidase was also associated with eNOS uncoupling.

The proof of the concept that superoxide produced by the NADPH oxidase may indeed trigger eNOS uncoupling was provided by David Harrison's group in the experimental animal model of DOCA-salt hypertension (39). With these studies, the authors showed that superoxide induced by DOCA-salt treatment caused increased vascular $H_2O_2$ production, which was significantly reduced by the eNOS inhibitor $N^G$-nitro-L-arginine methylester (L-NAME). Treatment of p47phox knockout animals with DOCA-salt markedly reduced levels of oxidative stress and abolished superoxide reducing effects of NOS inhibition compatible with a prevention of eNOS uncoupling (39) (Fig. 6).

## ASSESSMENT OF ENOS UNCOUPLING IN VASCULAR TISSUE

It is important to note that eNOS-mediated superoxide production—by the isolated enzyme or in vascular tissue—is inhibited by $N^G$-nitro-L-arginine (L-NNA) or its methylester $N^G$-nitro-L-arginine methylester (L-NAME), since both substances antagonize the transfer of electrons to either L-arginine or oxygen. In contrast, L-NMMA has been previously shown to stimulate rather than to inhibit superoxide production by the isolated enzyme, due to causing partial uncoupling of NADPH oxidation (electron transfer to oxygen). This has been shown for inducible NOS (iNOS) (54) and for neuronal NOS (nNOS) (60). Similar phenomena may have to be expected when isolated enzymes are exposed to the structurally similar asymmetric dimethyl-L-arginine (ADMA), which will prevent the

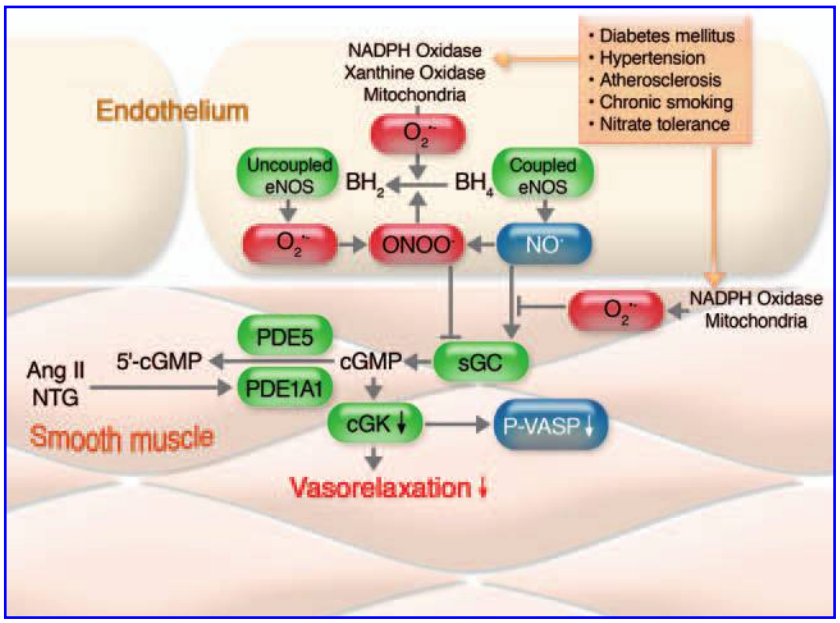


**FIG. 7. Schematic representation of the role of reactive oxygen species in causing endothelial dysfunction.** Under normal conditions, nitric oxide (NO), synthesized by nitric oxide synthase (eNOS), stimulates soluble guanylate cyclase (sGC), increasing cGMP and thus stimulating cGMP-dependent protein kinase I (cGK-I) and vasorelaxation. cGK-I also phosphorylates the vasodilator-stimulated phosphoprotein (VASP), which can be taken as a biochemical parameter of cGK-I activity. This pathway can, however, be inhibited at several sites. Angiotensin II, hypertension, hypercholesterolemia, chronic smoking, and diabetes mellitus will stimulate superoxide production within endothelial and smooth muscle cells mainly via activation of the vascular NADPH oxidase. The NADPH oxidase-derived superoxide may inactivate NO and inhibit sGC directly, thereby diminishing cGK-I activity. Peroxynitrite ($ONOO^-$) may also inhibit sGC directly, and it uncouples eNOS by oxidizing the eNOS cofactor to the $BH_3$. radical and $BH_2$, respectively. This concept, however, also means that uncoupling of eNOS would always require a "priming event" such as superoxide produced by the NADPH oxidase, mitochondria, or the xanthine oxidase (kindling radical). Angiotensin II also increases the catabolism of cGMP by the phosphodiesterase 1A1 (PDE1A1), which is usually accomplished by the phosphodiesterase 5 (adapted from ref. 4). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

oxidation of L-arginine and therefore, reduce NO production, or as mentioned above, will stimulate superoxide production by competing for the same binding site of the enzyme. Up to now, however, evidence that ADMA can trigger eNOS uncoupling is lacking.

The L-NNA sensitive and L-NAME sensitive inhibition of superoxide formation is a convenient method to detect eNOS uncoupling in vascular tissues. Depending on the detection system used, addition of NOS inhibitors to vascular cells will decrease the superoxide derived signal, such as lucigenin-enhanced chemiluminescence (5 $\mu$mol/L) (71) or the dihydroethidine fluorescence (29, 46). The group of David Harrison showed that depletion of endogenous $BH_4$ in mouse aorta by peroxynitrite increased superoxide production and that the addition of L-NAME as well as endothelial removal or genetic knockout of eNOS prevented this radical response (39).

## ENOS UNCOUPLING AND DOWNSTREAM SIGNALING BY THE SOLUBLE GUANYLYL CYCLASE (SGC) AND CGMP-DEPENDENT PROTEIN KINASE (CGK-I) IN HYPERTENSION

### *P-VASP, a novel marker suited to assess the activity of the cGK-I*

cGK-I activity can be assessed by immunodetection of phosphorylation at Serine239 of the cGK-I substrate vasodilator-stimulated phosphoprotein (P-VASP). This approach is a reliable surrogate parameter of the integrity of the NO/cGMP signaling pathway in platelets, cultured endothelial and smooth muscle cells (72, 73), and as shown more recently, also in intact vascular tissues (31, 52). The P-VASP assay appears to be sufficiently sensitive to monitor basal NO release in endothelium-intact arteries. For example, incubation of vessels with the NOS inhibitors L-NNA or L-NAME strongly decreased P-VASP levels (31, 52). Likewise, NO donors such as sodium nitroprusside (SNP) and nitroglycerin (NTG) markedly increased P-VASP in vessels in the absence and presence of an intact endothelium (31, 52). Mechanical removal of the endothelium in rat and rabbit aorta almost completely abolished "basal" VASP phosphorylation, whereas total VASP levels (phosphorylated and non-phosphorylated) were reduced by ~40–50% (31, 52). These findings indicate that VASP Serine239 phosphorylation clearly depends on the presence of a functional endothelium, and that the endothelial cell layer in aortic vessels contains a considerable amount of VASP, which is lost upon removal of the endothelium. P-VASP analysis also appears suitable to monitor oxidative stress within vascular tissue (Fig. 7). Incubation of isolated endothelium-intact vessels with diethylthiocarbamate (DETC), an inhibitor of Cu/Zn superoxide dismutase (SOD), markedly reduced P-VASP levels (52). Concomitantly, basal endothelial NO formation was decreased and formation of superoxide was increased (52).

## ENOS UNCOUPLING AND CONSEQUENCES FOR THE ACTIVITY AND EXPRESSION OF THE SGC IN HYPERTENSION

Endothelial dysfunction in different animal models of hypertension has been shown to be associated with increased expres-

sion of eNOS (44, 47) but decreased vascular NO bioavailability (47). Uncoupled eNOS (47) and NADPH oxidase (72) were identified as significant superoxide sources by the observation that NOS inhibition decreased superoxide production in the tissue from hypertensive animals (47) and by the observed increases in expression and enzyme activity of NADPH oxidase subunits nox1, p22phox, p67phox, and gp91phox (8, 47, 63). Increased superoxide production involved all vessel layers, namely the endothelium, media, and adventitia (47), and may also increase vasoconstrictor tone via stimulation of the expression of endothelin-1 in the smooth muscle and endothelial cell layers (34, 35). Acute or chronic treatment with antioxidants or superoxide dismutase not only improved endothelial dysfunction but also markedly reduced blood pressure indicating the potential role of ROS in the initiation and maintenance of hypertension.

Hypertensive animals displayed not only reduced vasodilation to endothelium-dependent vasodilators but also to endothelium-independent nitro-vasodilators SNP and NTG (63). This finding may be explained at least in part by reduced expression of sGC since in different hypertensive animal models the expression of one or both sGC subunits ($\alpha_1$ and $\beta_1$) as well as NO-dependent sGC activity was significantly decreased. This was observed in genetic hypertension, such as aged SHR (37, 65), stroke-prone SHR (43), type 2 diabetic, mildly hypertensive Goto–Kakizaki rats (88), and TGR(mREN2)27 renin transgenic rats (33), as well as in several models of drug-induced hypertension.

Reduction of hypertension normalized or even enhanced sGC expression, but had differing effects on vascular superoxide production. For example, in the angiotensin II-infusion model, inhibition of protein kinase C (PKC) *in vivo* reduced blood pressure and vascular superoxide formation by inhibiting the activity and expression of NADPH oxidase and by preventing eNOS uncoupling (47) and sGC downregulation (unpublished observation). In contrast, other forms of antihypertensive treatment (*e.g.*, with hydralazine), significantly lowered blood pressure but failed to normalize vascular superoxide formation in chronically NOS-inhibited rats (2). Thus, it appears that the decrease in sGC expression elicited by high blood pressure is not exclusively linked to increased superoxide formation and/or to increased levels of vasoconstrictor peptides. In contrary, vasoconstrictor peptides such as angiotensin II or endothelin-1, which are increased in hypertension, seem to trigger an upregulation of sGC in vascular smooth muscle cells. This sGC upregulation is apparently overwhelmed by effects of yet undetermined mediators of hypertension, which decrease sGC expression and NO/cGMP signaling, thereby promoting endothelial and smooth muscle dysfunction.

cGK I expression in aortic tissue was not changed in two different models of hypertension. Still, cGK I activity assessed by P-VASP analysis was markedly reduced and suggestive of a signaling defect upstream of cGK-I (47). In support of this, treatment with chelerythrine, an inhibitor of protein kinase C, reduced oxidative stress, increased P-VASP formation, and improved endothelial dysfunction (unpublished observation). *In vitro*, angiotensin II increased the activity and expression of the cGMP-specific PDE1A1 isoform in cultured smooth muscle cells. Thus, we can not exclude that increased cGMP metabolism may contribute partly to the endothelial dysfunction and decreased cGK-I activity observed in angiotensin II induced hypertension (36). (Fig. 7).

## SUMMARY AND CONCLUSIONS

Taken together, there is mounting evidence that endothelial dysfunction of the coronary or peripheral circulation has important prognostic implications for future cardiovascular events in patients with coronary artery disease, peripheral vascular disease, essential hypertension, or congestive heart failure. Although the mechanisms underlying endothelial dysfunction are likely multifactorial, it is important to note that increased production of oxygen-derived free radicals by an uncoupled eNOS markedly contributes to this phenomenon. One of the key mechanisms causing eNOS uncoupling is attributed to a decrease in intracellular $BH_4$ levels caused either by peroxynitrite-induced $BH_4$ oxidation or by decreased activity and/or expression of the GTP CH-I and the DHFR.

Increased superoxide production is not restricted to the endothelium and also involves the smooth muscle cell layer, and may lead to an alteration of the expression of the sGC and to an inhibition of the activity of sGC and subsequently cGK-I. The inhibition and/or activation of cGK-I in vascular tissue can be perfectly monitored by quantifying the degree of phosphorylation of VASP, a new tool which reliably reflects vascular NO bioavailability.

## ABBREVIATIONS

ACE, angiotensin converting enzyme; Ach, acetylcholine; ADMA, asymmetric dimethyl-L-arginine; $BH_4$, tetrahydrobiopterin; cGKI, cGMP dependent proteinkinase; cGMP, cyclic guanosine monophosphate; CTP CH-I, GTP cyclohydrolase I; DDAH, dimethylarginine dimethylaminohydrolase; DETC, diethylthiocarbamate; DHFR, dihydrofolate reductase; DOCA, deoxycorticosterone acetate; ED, endothelial dysfunction; eNOS, endothelial nitric oxide synthase; FBF, forearm blood flow; FMD, flow-mediated dilation; iNOS, inducible nitric oxide synthase; LDL, low density lipoprotein; 5MTHF, 5-methyl tetrahydrofolate; L-NAME, $N^G$-nitro-L-arginine methylester; L-NNA, $N^G$-nitro-L-arginine; LVMI, left ventricular mass index; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NTG, nitroglycerin; $ONOO^-$, peroxynitrite; PDE1A1, phosphodiesterase 1A1; PKC, protein kinase C, PRMT, protein arginine *N*-methyltransferase, ROS, reactive oxygen species; sGC, soluble guanylyl cyclase; SHR, spontaneously hypertensive rat; SNP, sodium nitroprusside; SOD, superoxide dismutase; STZ, streptozotocin; VASP, vasodilator-stimulated phosphoproteins.

## REFERENCES

1. Bastian NR and Hibbs JB, Jr. Assembly and regulation of NADPH oxidase and nitric oxide synthase. *Curr Opin Immunol* 6: 131–139, 1994.
2. Bauersachs J, Bouloumie A, Fraccarollo D, Hu K, Busse R, and Ertl G. Hydralazine prevents endothelial dysfunction, but not the increase in superoxide production in nitric oxide-deficient hypertension. *Eur J Pharmacol* 362: 77–81, 1998.
3. Beckman JS. Oxidative damage and tyrosine nitration from peroxynitrite. *Chem Res Toxicol* 9: 836–844, 1996.
4. Beckman JS and Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am J Physiol* 271: C1424–1437, 1996.

5. Bendall JK, Alp NJ, Warrick N, Cai S, Adlam D, Rockett K, Yokoyama M, Kawashima S, and Channon KM. Stoichiometric relationships between endothelial tetrahydrobiopterin, endothelial NO synthase (eNOS) activity, and eNOS coupling in vivo: insights from transgenic mice with endothelial-targeted GTP cyclohydrolase 1 and eNOS overexpression. *Circ Res* 97: 864–871, 2005.
6. Boger RH, Sydow K, Borlak J, Thum T, Lenzen H, Schubert B, Tsikas D, and Bode–Boger SM. LDL cholesterol upregulates synthesis of asymmetrical dimethylarginine in human endothelial cells: involvement of S-adenosylmethionine-dependent methyltransferases. *Circ Res* 87: 99–105, 2000.
7. Chalupsky K and 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 USA* 102: 9056–9061, 2005.
8. Cifuentes ME, Rey FE, Carretero OA, and Pagano PJ. Upregulation of p67(phox) and gp91(phox) in aortas from angiotensin II-infused mice. *Am J Physiol Heart Circ Physiol* 279: H2234–2240, 2000.
9. Closs EI, Scheld JS, Sharafi M, and Forstermann U. Substrate supply for nitric-oxide synthase in macrophages and endothelial cells: role of cationic amino acid transporters. *Mol Pharmacol* 57: 68–74, 2000.
10. Diet F, Pratt RE, Berry GJ, Momose N, Gibbons GH, and Dzau VJ. Increased accumulation of tissue ACE in human atherosclerotic coronary artery disease. *Circulation* 94: 2756–2767, 1996.
11. Drexler H, Zeiher AM, Meinzer K, and Just H. Correction of endothelial dysfunction in coronary microcirculation of hypercholesterolaemic patients by L-arginine. *Lancet* 338: 1546–1550, 1991.
12. Drummond GR, Cai H, Davis ME, Ramasamy S, and Harrison DG. Transcriptional and posttranscriptional regulation of endothelial nitric oxide synthase expression by hydrogen peroxide. *Circ Res* 86: 347–354, 2000.
13. Duffy SJ, Gokce N, Holbrook M, Hunter LM, Biegelsen ES, Huang A, Keaney JF, Jr., and Vita JA. Effect of ascorbic acid treatment on conduit vessel endothelial dysfunction in patients with hypertension. *Am J Physiol Heart Circ Physiol* 280: H528–534, 2001.
14. Forstermann U and Munzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. *Circulation* 113: 1708–1714, 2006.
15. Fukui T, Ishizaka N, Rajagopalan S, Laursen JB, Capers Qt, Taylor WR, Harrison DG, de Leon H, Wilcox JN, and Griendling KK. p22phox mRNA expression and NADPH oxidase activity are increased in aortas from hypertensive rats. *Circ Res* 80: 45–51, 1997.
16. Garg UC and Hassid A. Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *J Clin Invest* 83: 1774–1777, 1989.
17. Gokce N, Keaney JF, Jr., Hunter LM, Watkins MT, Menzoian JO, and Vita JA. Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. *Circulation* 105: 1567–1572, 2002.
18. Gokce N, Keaney JF, Jr., Hunter LM, Watkins MT, Nedeljkovic ZS, Menzoian JO, and Vita JA. Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. *J Am Coll Cardiol* 41: 1769–1775, 2003.
19. Gorren AC, List BM, Schrammel A, Pitters E, Hemmens B, Werner ER, Schmidt K, and Mayer B. Tetrahydrobiopterin-free neuronal nitric oxide synthase: evidence for two identical highly anticooperative pteridine binding sites. *Biochemistry* 35: 16735–16745, 1996.
20. Griendling KK, Sorescu D, and Ushio–Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. *Circ Res* 86: 494–501, 2000.
21. Gryglewski RJ, Moncada S, and Palmer RM. Bioassay of prostacyclin and endothelium-derived relaxing factor (EDRF) from porcine aortic endothelial cells. *Br J Pharmacol* 87: 685–694, 1986.
22. Guzik TJ, Mussa S, Gastaldi D, Sadowski J, Ratnatunga C, Pillai R, and Channon KM. Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. *Circulation* 105: 1656–1662, 2002.
23. Guzik TJ, West NE, Black E, McDonald D, Ratnatunga C, Pillai R, and Channon KM. Vascular superoxide production by NAD(P)H oxidase: association with endothelial dysfunction and clinical risk factors. *Circ Res* 86: E85–90, 2000.
24. Heitzer T, Baldus S, von Kodolitsch Y, Rudolph V, and Meinertz T. Systemic endothelial dysfunction as an early predictor of adverse outcome in heart failure. *Arterioscler Thromb Vasc Biol* 25: 1174–1179, 2005.
25. Heitzer T, Just H, and Munzel T. Antioxidant vitamin C improves endothelial dysfunction in chronic smokers. *Circulation* 94: 6–9, 1996.
26. Heitzer T, Krohn K, Albers S, and Meinertz T. Tetrahydrobiopterin improves endothelium-dependent vasodilation by increasing nitric oxide activity in patients with Type II diabetes mellitus. *Diabetologia* 43: 1435–1438, 2000.
27. Heitzer T, Schlinzig T, Krohn K, Meinertz T, and Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation* 104: 2673-2678, 2001.
28. Higashi Y, Sasaki S, Nakagawa K, Fukuda Y, Matsuura H, Oshima T, and Chayama K. Tetrahydrobiopterin enhances forearm vascular response to acetylcholine in both normotensive and hypertensive individuals. *Am J Hypertens* 15: 326–332, 2002.
29. 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, and Munzel T. Mechanisms underlying endothelial dysfunction in diabetes mellitus. *Circ Res* 88: E14–22, 2001.
30. Hishikawa K, Nakaki T, Suzuki H, Kato R, and Saruta T. Role of L-arginine-nitric oxide pathway in hypertension. *J Hypertens* 11: 639–645, 1993.
31. Ibarra–Alvarado C, Galle J, Melichar VO, Mameghani A, and Schmidt HH. Phosphorylation of blood vessel vasodilator-stimulated phosphoprotein at serine 239 as a functional biochemical marker of endothelial nitric oxide/cyclic GMP signaling. *Mol Pharmacol* 61: 312–319, 2002.
32. Imaizumi T, Hirooka Y, Masaki H, Harada S, Momohara M, Tagawa T, and Takeshita A. Effects of L-arginine on forearm vessels and responses to acetylcholine. *Hypertension* 20: 511–517, 1992.
33. Jacke K, Witte K, Huser L, Behrends S, and Lemmer B. Contribution of the renin-angiotensin system to subsensitivity of soluble guanylyl cyclase in TGR(mREN2)27 rats. *Eur J Pharmacol* 403: 27–35, 2000.
34. Kahler J, Ewert A, Weckmuller J, Stobbe S, Mittmann C, Koster R, Paul M, Meinertz T, and Munzel T. Oxidative stress increases endothelin-1 synthesis in human coronary artery smooth muscle cells. *J Cardiovasc Pharmacol* 38: 49–57, 2001.
35. Kahler J, Mendel S, Weckmuller J, Orzechowski HD, Mittmann C, Koster R, Paul M, Meinertz T, and Munzel T. Oxidative stress increases synthesis of big endothelin-1 by activation of the endothelin-1 promoter. *J Mol Cell Cardiol* 32: 1429–1437, 2000.
36. Kim D, Rybalkin SD, Pi X, Wang Y, Zhang C, Munzel T, Beavo JA, Berk BC, and Yan C. Upregulation of phosphodiesterase 1A1 expression is associated with the development of nitrate tolerance. *Circulation* 104: 2338–2343, 2001.
37. Kloss S, Bouloumie A, and Mulsch A. Aging and chronic hypertension decrease expression of rat aortic soluble guanylyl cyclase. *Hypertension* 35: 43–47, 2000.
38. Kuzkaya N, Weissmann N, Harrison DG, and Dikalov S. Interactions of peroxynitrite, tetrahydrobiopterin, ascorbic acid, and thiols: implications for uncoupling endothelial nitric-oxide synthase. *J Biol Chem* 278: 22546–22554, 2003.
39. Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, Mitch WE, and Harrison DG. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J Clin Invest* 111: 1201–1209, 2003.
40. Laursen JB, Somers M, Kurz S, McCann L, Warnholtz A, Freeman BA, Tarpey M, Fukai T, and Harrison DG. Endothelial regulation of vasomotion in apoE-deficient mice: implications for interactions between peroxynitrite and tetrahydrobiopterin. *Circulation* 103: 1282–1288, 2001.

41. Levine GN, Frei B, Koulouris SN, Gerhard MD, Keaney JF, Jr., and Vita JA. Ascorbic acid reverses endothelial vasomotor dysfunction in patients with coronary artery disease. *Circulation* 93: 1107–1113, 1996.
42. Lin KY, Ito A, Asagami T, Tsao PS, Adimoolam S, Kimoto M, Tsuji H, Reaven GM, and Cooke JP. Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylarginine dimethylaminohydrolase. *Circulation* 106: 987–992, 2002.
43. Lopez–Farre A, Rodriguez–Feo JA, Garcia–Colis E, Gomez J, Lopez–Blaya A, Fortes J, de Andres R, Rico L, and Casado S. Reduction of the soluble cyclic GMP vasorelaxing system in the vascular wall of stroke-prone spontaneously hypertensive rats: effect of the alpha1 -receptor blocker doxazosin. *J Hypertens* 20: 463–470, 2002.
44. Marques M, Millas I, Jimenez A, Garcia–Colis E, Rodriguez–Feo JA, Velasco S, Barrientos A, Casado S, and Lopez–Farre A. Alteration of the soluble guanylate cyclase system in the vascular wall of lead-induced hypertension in rats. *J Am Soc Nephrol* 12: 2594–2600, 2001.
45. Martasek P, Miller RT, Liu Q, Roman LJ, Salerno JC, Migita CT, Raman CS, Gross SS, Ikeda–Saito M, and Masters BS. The C331A mutant of neuronal nitric-oxide synthase is defective in arginine binding. *J Biol Chem* 273: 34799–34805, 1998.
46. Mollnau H, Oelze M, August M, Wendt M, Daiber A, Schulz E, Baldus S, Kleschyov AL, Materne A, Wenzel P, Hink U, Nickenig G, Fleming I, and Munzel T. Mechanisms of increased vascular superoxide production in an experimental model of idiopathic dilated cardiomyopathy. *Arterioscler Thromb Vasc Biol* 25: 2554–2559, 2005.
47. Mollnau H, Wendt M, Szocs K, Lassegue B, Schulz E, Oelze M, Li H, Bodenschatz M, August M, Kleschyov AL, Tsilimingas N, Walter U, Forstermann U, Meinertz T, Griendling K, and Munzel T. Effects of angiotensin II infusion on the expression and function of NAD(P)H oxidase and components of nitric oxide/cGMP signaling. *Circ Res* 90: E58–65, 2002.
48. Morawietz H, Weber M, Rueckschloss U, Lauer N, Hacker A, and Kojda G. Upregulation of vascular NAD(P)H oxidase subunit gp91phox and impairment of the nitric oxide signal transduction pathway in hypertension. *Biochem Biophys Res Commun* 285: 1130–1135, 2001.
49. Munzel T, Daiber A, Ullrich V, and Mulsch A. Vascular consequences of endothelial nitric oxide synthase uncoupling for the activity and expression of the soluble guanylyl cyclase and the cGMP-dependent protein kinase. *Arterioscler Thromb Vasc Biol* 25: 1551–1557, 2005.
50. Myers PR, Minor RL, Jr., Guerra R, Jr., Bates JN, and Harrison DG. Vasorelaxant properties of the endothelium-derived relaxing factor more closely resemble S-nitrosocysteine than nitric oxide. *Nature* 345: 161–163, 1990.
51. Nickenig G, Baumer AT, Temur Y, Kebben D, Jockenhovel F, and Bohm M. Statin-sensitive dysregulated AT1 receptor function and density in hypercholesterolemic men. *Circulation* 100: 2131–2134, 1999.
52. Oelze M, Mollnau H, Hoffmann N, Warnholtz A, Bodenschatz M, Smolenski A, Walter U, Skatchkov M, Meinertz T, and Munzel T. Vasodilator-stimulated phosphoprotein serine 239 phosphorylation as a sensitive monitor of defective nitric oxide/cGMP signaling and endothelial dysfunction. *Circ Res* 87: 999–1005, 2000.
53. Ohishi M, Ueda M, Rakugi H, Naruko T, Kojima A, Okamura A, Higaki J, and Ogihara T. Enhanced expression of angiotensin-converting enzyme is associated with progression of coronary atherosclerosis in humans. *J Hypertens* 15: 1295–1302, 1997.
54. Olken NM and Marletta MA. NG-methyl-L-arginine functions as an alternate substrate and mechanism-based inhibitor of nitric oxide synthase. *Biochemistry* 32: 9677–9685, 1993.
55. Ozaki M, Kawashima S, Yamashita T, Hirase T, Namiki M, Inoue N, Hirata K, Yasui H, Sakurai H, Yoshida Y, Masada M, and Yokoyama M. Overexpression of endothelial nitric oxide synthase accelerates atherosclerotic lesion formation in apoE-deficient mice. *J Clin Invest* 110: 331–340, 2002.
56. Palmer RM, Ferrige AG, and Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 327: 524–526, 1987.
57. Perticone F, Ceravolo R, Pujia A, Ventura G, Iacopino S, Scozzafava A, Ferraro A, Chello M, Mastroroberto P, Verdecchia P, and Schillaci G. Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation* 104: 191–196, 2001.
58. Pieper GM. Acute amelioration of diabetic endothelial dysfunction with a derivative of the nitric oxide synthase cofactor, tetrahydrobiopterin. *J Cardiovasc Pharmacol* 29: 8–15, 1997.
59. Pollock JS, Forstermann U, Mitchell JA, Warner TD, Schmidt HH, Nakane M, and Murad F. Purification and characterization of particulate endothelium-derived relaxing factor synthase from cultured and native bovine aortic endothelial cells. *Proc Natl Acad Sci USA* 88: 10480–10484, 1991.
60. Pou S, Keaton L, Surichamorn W, and Rosen GM. Mechanism of superoxide generation by neuronal nitric-oxide synthase. *J Biol Chem* 274: 9573–9580, 1999.
61. Pritchard KA, Jr., Groszek L, Smalley DM, Sessa WC, Wu M, Villalon P, Wolin MS, and Stemerman MB. Native low-density lipoprotein increases endothelial cell nitric oxide synthase generation of superoxide anion. *Circ Res* 77: 510–518, 1995.
62. Radomski MW, Palmer RM, and Moncada S. The anti-aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. *Br J Pharmacol* 92: 639–646, 1987.
63. Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griendling KK, and Harrison DG. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. *J Clin Invest* 97: 1916–1923, 1996.
64. Rossitch E, Jr., Alexander E, 3rd, Black PM, and Cooke JP. L-arginine normalizes endothelial function in cerebral vessels from hypercholesterolemic rabbits. *J Clin Invest* 87: 1295–1299, 1991.
65. Ruetten H, Zabel U, Linz W, and Schmidt HH. Downregulation of soluble guanylyl cyclase in young and aging spontaneously hypertensive rats. *Circ Res* 85: 534–541, 1999.
66. Schachinger V, Britten MB, and Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* 101: 1899–1906, 2000.
67. Schieffer B, Schieffer E, Hilfiker–Kleiner D, Hilfiker A, Kovanen PT, Kaartinen M, Nussberger J, Harringer W, and Drexler H. Expression of angiotensin II and interleukin 6 in human coronary atherosclerotic plaques: potential implications for inflammation and plaque instability. *Circulation* 101: 1372–1378, 2000.
68. Schmidt TS and Alp NJ. Mechanisms for the role of tetrahydrobiopterin in endothelial function and vascular disease. *Clin Sci (Lond)* 113: 47–63, 2007.
69. Sciacqua A, Scozzafava A, Pujia A, Maio R, Borrello F, Andreozzi F, Vatrano M, Cassano S, Perticone M, Sesti G, and Perticone F. Interaction between vascular dysfunction and cardiac mass increases the risk of cardiovascular outcomes in essential hypertension. *Eur Heart J* 26: 921–927, 2005.
70. Setoguchi S, Mohri M, Shimokawa H, and Takeshita A. Tetrahydrobiopterin improves endothelial dysfunction in coronary microcirculation in patients without epicardial coronary artery disease. *J Am Coll Cardiol* 38: 493–498, 2001.
71. Skatchkov MP, Sperling D, Hink U, Anggard E, and Munzel T. Quantification of superoxide radical formation in intact vascular tissue using a *Cypridina luciferin* analog as an alternative to lucigenin. *Biochem Biophys Res Commun* 248: 382–386, 1998.
72. Smolenski A, Bachmann C, Reinhard K, Honig-Liedl P, Jarchau T, Hoschuetzky H, and Walter U. Analysis and regulation of vasodilator-stimulated phosphoprotein serine 239 phosphorylation in vitro and in intact cells using a phosphospecific monoclonal antibody. *J Biol Chem* 273: 20029–20035, 1998.
73. Smolenski A, Burkhardt AM, Eigenthaler M, Butt E, Gambaryan S, Lohmann SM, and Walter U. Functional analysis of cGMP-dependent protein kinases I and II as mediators of NO/cGMP effects. *Naunyn Schmiedebergs Arch Pharmacol* 358: 134–139, 1998.
74. Sorescu D, Weiss D, Lassegue B, Clempus RE, Szocs K, Sorescu GP, Valppu L, Quinn MT, Lambeth JD, Vega JD, Taylor WR, and Griendling KK. Superoxide production and expression of nox family proteins in human atherosclerosis. *Circulation* 105: 1429–1435, 2002.
75. Stroes E, Kastelein J, Cosentino F, Erkelens W, Wever R, Koomans H, Luscher T, and Rabelink T. Tetrahydrobiopterin restores endo-

thelial function in hypercholesterolemia. *J Clin Invest* 99: 41–46, 1997.
76. Stroes ES, van Faassen EE, Yo M, Martasek P, Boer P, Govers R, and Rabelink TJ. Folic acid reverts dysfunction of endothelial nitric oxide synthase. *Circ Res* 86: 1129–1134, 2000.
77. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Jr., and Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation* 101: 948–954, 2000.
78. Sydow K and Munzel T. ADMA and oxidative stress. *Atheroscler Suppl* 4: 41–51, 2003.
79. Sydow K, Schwedhelm E, Arakawa N, Bode–Boger SM, Tsikas D, Hornig B, Frolich JC, and Boger RH. ADMA and oxidative stress are responsible for endothelial dysfunction in hyperhomocyst(e)inemia: effects of L-arginine and B vitamins. *Cardiovasc Res* 57: 244–252, 2003.
80. Ting HH, Timimi FK, Boles KS, Creager SJ, Ganz P, and Creager MA. Vitamin C improves endothelium-dependent vasodilation in patients with non-insulin-dependent diabetes mellitus. *J Clin Invest* 97: 22–28, 1996.
81. Vasquez–Vivar J, Kalyanaraman B, Martasek P, Hogg N, Masters BS, Karoui H, Tordo P, and Pritchard KA, Jr. Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors. *Proc Natl Acad Sci USA* 95: 9220–9225, 1998.
82. Vasquez–Vivar J, Martasek P, Whitsett J, Joseph J, and Kalyanaraman B. The ratio between tetrahydrobiopterin and oxidized tetrahydrobiopterin analogues controls superoxide release from endothelial nitric oxide synthase: an EPR spin trapping study. *Biochem J* 362: 733–739, 2002.
83. Vaziri ND, Ni Z, and Oveisi F. Upregulation of renal and vascular nitric oxide synthase in young spontaneously hypertensive rats. *Hypertension* 31: 1248–1254, 1998.
84. Vergnani L, Hatrik S, Ricci F, Passaro A, Manzoli N, Zuliani G, Brovkovych V, Fellin R, and Malinski T. Effect of native and oxidized low-density lipoprotein on endothelial nitric oxide and superoxide production : key role of L-arginine availability. *Circulation* 101: 1261–1266, 2000.
85. Verhaar MC, Stroes E, and Rabelink TJ. Folates and cardiovascular disease. *Arterioscler Thromb Vasc Biol* 22: 6–13, 2002.
86. Verhaar MC, Wever RM, Kastelein JJ, van Dam T, Koomans HA, and Rabelink TJ. 5-methyltetrahydrofolate, the active form of folic acid, restores endothelial function in familial hypercholesterolemia. *Circulation* 97: 237–241, 1998.
87. Warnholtz A, Nickenig G, Schulz E, Macharzina R, Brasen JH, Skatchkov M, Heitzer T, Stasch JP, Griendling KK, Harrison DG, Bohm M, Meinertz T, and Munzel T. Increased NADH-oxidase-mediated superoxide production in the early stages of atherosclerosis: evidence for involvement of the renin- angiotensin system. *Circulation* 99: 2027–2033, 1999.
88. Witte K, Jacke K, Stahrenberg R, Arlt G, Reitenbach I, Schilling L, and Lemmer B. Dysfunction of soluble guanylyl cyclase in aorta and kidney of Goto-Kakizaki rats: influence of age and diabetic state. *Nitric Oxide* 6: 85–95, 2002.
89. Xia Y and Zweier JL. Direct measurement of nitric oxide generation from nitric oxide synthase. *Proc Natl Acad Sci USA* 94: 12705–12710, 1997.
90. Zhang L, Rao F, Zhang K, Khandrika S, Das M, Vaingankar SM, Bao X, Rana BK, Smith DW, Wessel J, Salem RM, Rodriguez–Flores JL, Mahata SK, Schork NJ, Ziegler MG, and O'-Connor DT. Discovery of common human genetic variants of GTP cyclohydrolase 1 (GCH1) governing nitric oxide, autonomic activity, and cardiovascular risk. *J Clin Invest* 117: 2658–2671, 2007.
91. Zou MH, Shi C, and Cohen RA. Oxidation of the zinc-thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite. *J Clin Invest* 109: 817–826, 2002.
92. Zou MH and Ullrich V. Peroxynitrite formed by simultaneous generation of nitric oxide and superoxide selectively inhibits bovine aortic prostacyclin synthase. *FEBS Lett* 382: 101–104, 1996.

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3. Manuel Maglione, Benno Cardini, Rupert Oberhuber, Katrin Watschinger, Marcel Jenny, Johanna Gostner, Martin Hermann, Peter Obrist, Raimund Margreiter, Johann Pratschke, Gerald Brandacher, Ernst R. Werner. 2012. Prevention of lethal murine pancreas ischemia reperfusion injury is specific for tetrahydrobiopterin. *Transplant International* **25**:10, 1084-1095. [CrossRef]
4. T. Hilton Grayson, Preet S. Chadha, Paul P. Bertrand, Hui Chen, Margaret J. Morris, Sevvandi Senadheera, Timothy V. Murphy, Shaun L. Sandow. 2012. Increased caveolae density and caveolin-1 expression accompany impaired NO-mediated vasorelaxation in diet-induced obesity. *Histochemistry and Cell Biology* . [CrossRef]
5. Anantha Vijay R. Santhanam, Livius V. d'Uscio, Leslie A. Smith, Zvonimir S. Katusic. 2012. Uncoupling of eNOS causes superoxide anion production and impairs NO signaling in the cerebral microvessels of hph-1 mice. *Journal of Neurochemistry* **122**:6, 1211-1218. [CrossRef]
6. Juliana G. Franco, Patrícia C. Lisboa, Natália S. Lima, Taline A.S. Amaral, Nayara Peixoto-Silva, Angela C. Resende, Elaine Oliveira, Magna C.F. Passos, Egberto G. Moura. 2012. Resveratrol attenuates oxidative stress and prevents steatosis and hypertension in obese rats programmed by early weaning. *The Journal of Nutritional Biochemistry* . [CrossRef]
7. Eberhard Schulz , Philip Wenzel , Thomas Münzel , Andreas Daiber . Mitochondrial Redox Signaling: Interaction of Mitochondrial Reactive Oxygen Species with Other Sources of Oxidative Stress. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
8. Pitchai Balakumar, Gaurav Taneja. 2012. Fish oil and vascular endothelial protection: Bench to bedside. *Free Radical Biology and Medicine* **53**:2, 271-279. [CrossRef]
9. Alex F. Chen, Dan#Dan Chen, Andreas Daiber, Frank M. Faraci, Huige Li, Christopher M. Rembold, Ismail Laher. 2012. Free radical biology of the cardiovascular system. *Clinical Science* **123**:2, 73-91. [CrossRef]
10. Ji Young Park, Jung Wook Yun, Young Whan Choi, Jin Ung Bae, Kyo Won Seo, Seung Jin Lee, So Youn Park, Ki Whan Hong, Chi Dae Kim. 2012. Antihypertensive effect of gomisin A from Schisandra chinensis on angiotensin II-induced hypertension via preservation of nitric oxide bioavailability. *Hypertension Research* . [CrossRef]
11. Gonzalo García, Isabel Serrano, Patricia Sánchez-Alonso, Manuel Rodríguez-Puyol, Ramón Alajarín, Mercedes Griera, Juan J. Vaquero, Diego Rodríguez-Puyol, Julio Álvarez-Builla, María L. Díez-Marqués. 2012. New losartan-hydrocaffeic acid hybrids as antihypertensive-antioxidant dual drugs: Ester, amide and amine linkers. *European Journal of Medicinal Chemistry* **50**, 90-101. [CrossRef]
12. Devon S. Svoboda, Michael D. Kawaja. 2012. Changes in hepatic protein expression in spontaneously hypertensive rats suggest early stages of non-alcoholic fatty liver disease. *Journal of Proteomics* . [CrossRef]
13. Difei Bian, Mei Liu, Ying Li, Yufeng Xia, Zhunan Gong, Yue Dai. 2012. Madecassoside, a triterpenoid saponin isolated from Centella asiatica herbs, protects endothelial cells against oxidative stress. *Journal of Biochemical and Molecular Toxicology* n/a-n/a. [CrossRef]
14. David C. Wheeler, Richard Haynes, Martin J. Landray, Colin BaigentCardiovascular Aspects of Kidney Disease 2059-2080. [CrossRef]
15. H.-H. Hou, B. D. Hammock, K.-H. Su, C. Morisseau, Y. R. Kou, S. Imaoka, A. Oguro, S.-K. Shyue, J.-F. Zhao, T.-S. Lee. 2011. N-terminal domain of soluble epoxide hydrolase negatively regulates the VEGF-mediated activation of endothelial nitric oxide synthase. *Cardiovascular Research* . [CrossRef]
16. John A. Watts, Michael A. Gellar, Mary-Beth K. Fulkerson, Sudeep K. Das, Jeffrey A. Kline. 2011. Arginase depletes plasma l-arginine and decreases pulmonary vascular reserve during experimental pulmonary embolism. *Pulmonary Pharmacology & Therapeutics* . [CrossRef]
17. Peter R. Kvietys, D. Neil Granger. 2011. Role of reactive oxygen and nitrogen species in the vascular responses to inflammation. *Free Radical Biology and Medicine* . [CrossRef]
18. Hajime Otani . 2011. Oxidative Stress as Pathogenesis of Cardiovascular Risk Associated with Metabolic Syndrome. *Antioxidants & Redox Signaling* **15**:7, 1911-1926. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]

19. Kerry-Anne A. Perkins, Sailesh Pershad, Qian Chen, Sloane McGraw, Jovan S. Adams, Christopher Zambrano, Samuel Krass, Jeffrey Emrich, Brandon Bell, Michael Iyamu, Catherine Prince, Helen Kay, Jane Chun-wen Teng, Lindon H. Young. 2011. The effects of modulating eNOS activity and coupling in ischemia/reperfusion (I/R). *Naunyn-Schmiedeberg's Archives of Pharmacology* . [CrossRef]
20. Greg A. Knock , Jeremy P.T. Ward . 2011. Redox Regulation of Protein Kinases as a Modulator of Vascular Function. *Antioxidants & Redox Signaling* **15**:6, 1531-1547. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
21. Michel Félétou, Ralf Köhler, Paul M. Vanhoutte. 2011. Nitric oxide: Orchestrator of endothelium-dependent responses. *Annals of Medicine* 1-23. [CrossRef]
22. Barbara Mlinar, Janja Marc. 2011. Review: New insights into adipose tissue dysfunction in insulin resistance. *Clinical Chemistry and Laboratory Medicine* ---. [CrossRef]
23. Felix Fleissner , Thomas Thum . 2011. Critical Role of the Nitric Oxide/Reactive Oxygen Species Balance in Endothelial Progenitor Dysfunction. *Antioxidants & Redox Signaling* **15**:4, 933-948. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
24. Xiu-ying Yang, Lan Sun, Pei Xu, Li-li Gong, Gui-fen Qiang, Li Zhang, Guan-hua Du. 2011. Effects of salvianolic scid A on plantar microcirculation and peripheral nerve function in diabetic rats. *European Journal of Pharmacology* **665**:1-3, 40-46. [CrossRef]
25. Rafa# Por#ba, Pawe# Ga#, Ma#gorzata Por#ba, Jolanta Antonowicz-Juchniewicz, Ryszard Andrzejak. 2011. Relationship between occupational exposure to lead and local arterial stiffness and left ventricular diastolic function in individuals with arterial hypertension. *Toxicology and Applied Pharmacology* **254**:3, 342-348. [CrossRef]
26. David W. Brock, Connie L. Tompkins, Gordon Fisher, Gary R. Hunter. 2011. Influence of resting energy expenditure on blood pressure is independent of body mass and a marker of sympathetic tone. *Metabolism* . [CrossRef]
27. Marcelo F. Montenegro, Jefferson H. Amaral, Lucas C. Pinheiro, Eliana K. Sakamoto, Graziele C. Ferreira, Rosana Inacio Reis, Diogo M.O. Marçal, Romaiana P. Pereira, Jose E. Tanus-Santos. 2011. Sodium nitrite downregulates vascular NADPH oxidase and exerts antihypertensive effects in hypertension. *Free Radical Biology and Medicine* **51**:1, 144-152. [CrossRef]
28. Michel Félétou. 2011. The Endothelium, Part I: Multiple Functions of the Endothelial Cells -- Focus on Endothelium-Derived Vasoactive Mediators. *Colloquium Series on Integrated Systems Physiology: From Molecule to Function* **3**:4, 1-306. [CrossRef]
29. Michel Félétou. 2011. The Endothelium, Part II: EDHF-Mediated Responses "The Classical Pathway". *Colloquium Series on Integrated Systems Physiology: From Molecule to Function* **3**:4, 1-306. [CrossRef]
30. Rodrigo A. Loiola, Felipe C.G. Reis, Elisa M. Kawamoto, Cristoforo Scavone, Dulcinéia S. Abdalla, Liliam Fernandes, João Bosco Pesquero. 2011. Role of vascular Kinin B1 and B2 receptors in endothelial nitric oxide metabolism. *Peptides* . [CrossRef]
31. Jinsong Wang, XinZhi Peng, Roberta M. Lassance-Soares, Amir H. Najafi, Lee O. Alderman, Subeena Sood, Zhenyi Xue, Rosanna Chan, James E. Faber, Stephen E. Epstein, Mary Susan Burnett. 2011. Aging-Induced Collateral Dysfunction: Impaired Responsiveness of Collaterals and Susceptibility to Apoptosis via Dysfunctional eNOS signaling. *Journal of Cardiovascular Translational Research* . [CrossRef]
32. An L. Moens, Elizabeth A. Ketner, Eiki Takimoto, Tim S. Schmidt, Charles A. O'Neill, Michael S. Wolin, Nicholas J. Alp, Keith M. Channon, David A. Kass. 2011. Bi-modal dose-dependent cardiac response to tetrahydrobiopterin in pressure-overload induced hypertrophy and heart failure. *Journal of Molecular and Cellular Cardiology* . [CrossRef]
33. Christian Brinkmann, Robert H. G. Schwinger, Klara Brixius. 2011. Körperliche Aktivität und endotheliale Dysfunktion bei Typ-2-Diabetikern: über die Rolle von Stickstoffmonoxid und oxidativem Stress. *Wiener Medizinische Wochenschrift* . [CrossRef]
34. Xinghua Cheng , Richard C.M. Siow , Giovanni E. Mann . 2011. Impaired Redox Signaling and Antioxidant Gene Expression in Endothelial Cells in Diabetes: A Role for Mitochondria and the Nuclear Factor-E2-Related Factor 2-Kelch-Like ECH-Associated Protein 1 Defense Pathway. *Antioxidants & Redox Signaling* **14**:3, 469-487. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
35. K Wingler, JJR Hermans, P Schiffers, AL Moens, M Paul, HHHW Schmidt. 2011. NOX 1, 2, 4, 5: Counting out oxidative stress. *British Journal of Pharmacology* no-no. [CrossRef]
36. Clément Prati, Alain Berthelot, Daniel Wendling, Céline Demougeot. 2011. Endothelial dysfunction in rat adjuvant-induced arthritis: Upregulation of the vascular arginase pathway. *Arthritis & Rheumatism* n/a-n/a. [CrossRef]
37. Chiranjib Dasgupta, Lubo Zhang. 2011. Angiotensin II receptors and drug discovery in cardiovascular disease. *Drug Discovery Today* **16**:1-2, 22-34. [CrossRef]

38. Hyun-Ock Pae, Yong Son, Nam-Ho Kim, Hee Jong Jeong, Ki Churl Chang, Hun-Taeg Chung. 2010. Role of heme oxygenase in preserving vascular bioactive NO. *Nitric Oxide* **23**:4, 251-257. [CrossRef]
39. T. Munzel, T. Gori, R. M. Bruno, S. Taddei. 2010. Is oxidative stress a therapeutic target in cardiovascular disease?. *European Heart Journal* **31**:22, 2741-2748. [CrossRef]
40. M. Maglione, R. Oberhuber, B. Cardini, K. Watschinger, M. Hermann, P. Obrist, P. Hengster, W. Mark, S. Schneeberger, G. Werner-Felmayer, J. Pratschke, R. Margreiter, E. R. Werner, G. Brandacher. 2010. Donor Pretreatment with Tetrahydrobiopterin Saves Pancreatic Isografts from Ischemia Reperfusion Injury in a Mouse Model. *American Journal of Transplantation* **10**:10, 2231-2240. [CrossRef]
41. Su-li Zhang, Yun-hui Du, Jin Wang, Li-hong Yang, Xiao-li Yang, Rong-hua Zheng, Ye Wu, Ke Wang, Ming-sheng Zhang, Hui-rong Liu. 2010. Endothelial dysfunction induced by antibodies against angiotensin AT1 receptor in immunized rats. *Acta Pharmacologica Sinica* **31**:10, 1381-1388. [CrossRef]
42. Marcelo F. Montenegro, Evandro M. Neto-Neves, Carlos A. Dias-Junior, Carla S. Ceron, Michele M. Castro, Valeria A. Gomes, Alexandre Kanashiro, Jose E. Tanus-Santos. 2010. Quercetin restores plasma nitrite and nitroso species levels in renovascular hypertension. *Naunyn-Schmiedeberg's Archives of Pharmacology* **382**:4, 293-301. [CrossRef]
43. Aline C. Pereira, Vania C. Olivon, Ana M. de Oliveira. 2010. Impaired calcium influx despite hyper-reactivity in contralateral carotid following balloon injury: eNOS involvement. *European Journal of Pharmacology* **642**:1-3, 121-127. [CrossRef]
44. Melanie E Armitage, Mylinh La, Harald HHW Schmidt, Kirstin Wingler. 2010. Diagnosis and individual treatment of cardiovascular diseases: targeting vascular oxidative stress. *Expert Review of Clinical Pharmacology* **3**:5, 639-648. [CrossRef]
45. Michel Félétou, Ralf Köhler, Paul M. Vanhoutte. 2010. Endothelium-derived Vasoactive Factors and Hypertension: Possible Roles in Pathogenesis and as Treatment Targets. *Current Hypertension Reports* **12**:4, 267-275. [CrossRef]
46. Armin Hatzelmann, Esteban J. Morcillo, Giuseppe Lungarella, Serge Adnot, Shahin Sanjar, Rolf Beume, Christian Schudt, Hermann Tenor. 2010. The preclinical pharmacology of roflumilast – A selective, oral phosphodiesterase 4 inhibitor in development for chronic obstructive pulmonary disease. *Pulmonary Pharmacology & Therapeutics* **23**:4, 235-256. [CrossRef]
47. Jean-Christophe Lambry, Edward Beaumont, Bogdan Tarus, Mireille Blanchard-Desce, Anny Slama-Schwok. 2010. Selective probing of a NADPH site controlled light-induced enzymatic catalysis. *Journal of Molecular Recognition* **23**:4, 379-388. [CrossRef]
48. Michael A. Moskowitz, Eng H. Lo, Costantino Iadecola. 2010. The Science of Stroke: Mechanisms in Search of Treatments. *Neuron* **67**:2, 181-198. [CrossRef]
49. Dennis Schade, Jürke Kotthaus, Bernd Clement. 2010. Modulating the NO generating system from a medicinal chemistry perspective: Current trends and therapeutic options in cardiovascular disease. *Pharmacology & Therapeutics* **126**:3, 279-300. [CrossRef]
50. José L. Miguel-Carrasco, María T. Monserrat, Alfonso Mate, Carmen M. Vázquez. 2010. Comparative effects of captopril and l-carnitine on blood pressure and antioxidant enzyme gene expression in the heart of spontaneously hypertensive rats. *European Journal of Pharmacology* **632**:1-3, 65-72. [CrossRef]
51. Katsuhiko Noguchi, Naobumi Hamadate, Toshihiro Matsuzaki, Mayuko Sakanashi, Junko Nakasone, Makiko Sakanashi, Masato Tsutsui, Matao Sakanashi. 2010. Improvement of impaired endothelial function by tetrahydrobiopterin in stroke-prone spontaneously hypertensive rats. *European Journal of Pharmacology* **631**:1-3, 28-35. [CrossRef]
52. Modou O. Kane, Nelly Etienne-Selloum, Soccoro V. F. Madeira, Mamadou Sarr, Allison Walter, Stéphanie Dal-Ros, Christa Schott, Thierry Chataigneau, Valérie B. Schini-Kerth. 2010. Endothelium-derived contracting factors mediate the Ang II-induced endothelial dysfunction in the rat aorta: preventive effect of red wine polyphenols. *Pflügers Archiv - European Journal of Physiology* **459**:5, 671-679. [CrossRef]
53. Julien Amour, Anna K. Brzezinska, Zachary Jager, Corbin Sullivan, Dorothee Weihrauch, Jianhai Du, Nikolina Vladic, Yang Shi, David C. Warltier, Phillip F. Pratt, Judy R. Kersten. 2010. Hyperglycemia Adversely Modulates Endothelial Nitric Oxide Synthase during Anesthetic Preconditioning through Tetrahydrobiopterin– and Heat Shock Protein 90–mediated Mechanisms. *Anesthesiology* **112**:3, 576-585. [CrossRef]
54. Maya Sakurada, Takanobu Yoshimoto, Naoko Sekizawa, Yuki Hirono, Noriko Suzuki, Yukio Hirata. 2010. Vasculoprotective effect of cilostazol in aldosterone-induced hypertensive rats. *Hypertension Research* **33**:3, 229-235. [CrossRef]
55. S. Saidi, S. G. Mallat, W. Y. Almawi, T. Mahjoub. 2010. Endothelial nitric oxide synthase Glu298Asp, 4b/a, and â786T>C gene polymorphisms and the risk of ischemic stroke. *Acta Neurologica Scandinavica* **121**:2, 114-119. [CrossRef]
56. Rajesh Ramasamy, Maryam Maqbool, Abdul Latiff Mohamed, Rahim Md. Noah. 2010. Elevated neutrophil respiratory burst activity in essential hypertensive patients. *Cellular Immunology* **263**:2, 230-234. [CrossRef]

57. Kurt Schmidt, Margit Rehn, Heike Stessel, Gerald Wölkart, Bernd Mayer. 2010. Evidence against tetrahydrobiopterin depletion of vascular tissue exposed to nitric oxide/superoxide or nitroglycerin. *Free Radical Biology and Medicine* **48**:1, 145-152. [CrossRef]
58. Georgia Ragia, Eleftherios Nikolaidis, Anna Tavridou, Kostas I Arvanitidis, Stavroula Kanoni, George V Dedoussis, George Bougioukas, Vangelis G Manolopoulos. 2010. Endothelial nitric oxide synthase gene polymorphisms -786T >C and 894G >T in coronary artery bypass graft surgery patients. *Human Genomics* **4**:6, 375. [CrossRef]
59. A. E. Loot, J. G. Schreiber, B. Fisslthaler, I. Fleming. 2009. Angiotensin II impairs endothelial function via tyrosine phosphorylation of the endothelial nitric oxide synthase. *Journal of Experimental Medicine* **206**:13, 2889-2896. [CrossRef]
60. Jeffrey D. Laskin, Diane E. Heck, Debra L. LaskinNitric Oxide Pathways in Toxic Responses . [CrossRef]
61. Luciana Hannibal, Alla Glushchenko, Donald JacobsenFolate and Vascular Disease 291-323. [CrossRef]
62. Paul A. Fitzpatrick, Anthony F. Guinan, Tony G. Walsh, Ronan P. Murphy, Maria T. Killeen, Nicholas P. Tobin, Adrian R. Pierotti, Philip M. Cummins. 2009. Down-regulation of neprilysin (EC3.4.24.11) expression in vascular endothelial cells by laminar shear stress involves NADPH oxidase-dependent ROS production. *The International Journal of Biochemistry & Cell Biology* **41**:11, 2287-2294. [CrossRef]
63. Melanie E. Armitage, Kirstin Wingler, Harald H. H. W. Schmidt, Mylinh La. 2009. Translating the oxidative stress hypothesis into the clinic: NOX versus NOS. *Journal of Molecular Medicine* **87**:11, 1071-1076. [CrossRef]
64. Christoph A. Schmitt, Verena M. Dirsch. 2009. Modulation of endothelial nitric oxide by plant-derived products. *Nitric Oxide* **21**:2, 77-91. [CrossRef]
65. Ana B García-Redondo, Ana M Briones, María S Avendaño, Raquel Hernanz, María J Alonso, Mercedes Salaices. 2009. Losartan and tempol treatments normalize the increased response to hydrogen peroxide in resistance arteries from hypertensive rats. *Journal of Hypertension* **27**:9, 1814-1822. [CrossRef]
66. Olga Pechanova, Fedor Simko. 2009. Chronic antioxidant therapy fails to ameliorate hypertension: potential mechanisms behind. *Journal of Hypertension* **27**:Suppl 6, S32-S36. [CrossRef]
67. Qixuan Chen, Heidi Gruber, Eleonora Swist, Catherine Pakenham, Walisundera M. N. Ratnayake, Kylie A. Scoggan. 2009. Influence of dietary phytosterols and phytostanols on diastolic blood pressure and the expression of blood pressure regulatory genes in SHRSP and WKY inbred rats. *British Journal of Nutrition* **102**:01, 93. [CrossRef]
68. Emily J. Tsai, David A. Kass. 2009. Cyclic GMP signaling in cardiovascular pathophysiology and therapeutics. *Pharmacology & Therapeutics* **122**:3, 216-238. [CrossRef]
69. Joern R. Steinert , Amanda W. Wyatt , Ron Jacob , Giovanni E. Mann . 2009. Redox Modulation of Ca2+ Signaling in Human Endothelial and Smooth Muscle Cells in Pre-Eclampsia. *Antioxidants & Redox Signaling* **11**:5, 1149-1163. [Abstract] [Full Text PDF] [Full Text PDF with Links]
70. George Bakris. 2009. An In-depth Analysis of Vasodilation in the Management of Hypertension: Focus on Adrenergic Blockade. *Journal of Cardiovascular Pharmacology* **53**:5, 379-387. [CrossRef]
71. Priscilla R.V. Campana, Fernão C. Braga, Steyner F. Cortes. 2009. Endothelium-dependent vasorelaxation in rat thoracic aorta by Mansoa hirsuta D.C. *Phytomedicine* **16**:5, 456-461. [CrossRef]
72. Marcelo F. Montenegro, Lisandra R. Pessa, Valéria A. Gomes, Zeruesenay Desta, David A. Flockhart, Jose E. Tanus-Santos. 2009. Assessment of Vascular Effects of Tamoxifen and Its Metabolites on the Rat Perfused Hindquarter Vascular Bed. *Basic & Clinical Pharmacology & Toxicology* **104**:5, 400-407. [CrossRef]
73. Rasha H. Mohamed. 2009. Role of cilostazol in alleviating cardiovascular complications induced in experimental rats through regulation of type 1 plasminogen activator inhibitor and transforming growth factor-#1 overexpression. *Biomedicine & Pharmacotherapy* . [CrossRef]
74. Greg A. Knock, Vladimir A. Snetkov, Yasin Shaifta, Michelle Connolly, Svetlana Drndarski, Anthony Noah, Ghazaleh E. Pourmahram, Silke Becker, Philip I. Aaronson, Jeremy P.T. Ward. 2009. Superoxide constricts rat pulmonary arteries via Rho-kinase-mediated Ca2+ sensitization. *Free Radical Biology and Medicine* **46**:5, 633-642. [CrossRef]
75. Y SHAKIROVA, P HEDLUND, K SWARD. 2009. Impaired nerve-mediated relaxation of penile tissue from caveolin-1 deficient mice. *European Journal of Pharmacology* **602**:2-3, 399-405. [CrossRef]
76. Zvonimir S. Katusic, Livius V. d'Uscio, Karl A. Nath. 2009. Vascular protection by tetrahydrobiopterin: progress and therapeutic prospects. *Trends in Pharmacological Sciences* **30**:1, 48-54. [CrossRef]
77. C WUNDERLICH, K SCHOBER, C HEERWAGEN, R MARQUETANT, B EBNER, M FORKMANN, S SCHOEN, R BRAUNDULLAEUS, A SCHMEISSER, R STRASSER. 2008. Nitric oxide synthases are crucially involved in

the development of the severe cardiomyopathy of caveolin-1 knockout mice. *Biochemical and Biophysical Research Communications* **377**:3, 769-774. [CrossRef]

78. Eberhard Schulz, Thomas Münzel. 2008. NOX5, a New “Radical” Player in Human Atherosclerosis?##Editorials published in the Journal of the American College of Cardiology reflect the views of the authors and do not necessarily represent the views of JACC or the American College of Cardiology. *Journal of the American College of Cardiology* **52**:22, 1810-1812. [CrossRef]
79. B Mayer, M Beretta. 2008. The enigma of nitroglycerin bioactivation and nitrate tolerance: news, views and troubles. *British Journal of Pharmacology* **155**:2, 170-184. [CrossRef]