

Nitric Oxide and its Role in Cardiovascular Diseases

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Abstract: Nitric oxide synthases (NOS) are the enzymes responsible for nitric oxide (NO) generation. NO is a free radical which reacts with various molecules to cause multiple biological effects. It is clear that the generation and actions of NO under physiological and pathophysiological conditions are exquisitely regulated and extend to almost every cell type and function within the circulation. While the molecule mediates many physiological functions, an excessive presence of NO is toxic to cells. The enzyme NOS, constitutively or inductively, catalyses the production of NO in several biological systems. NO is derived not only from NOS isoforms but also from NOS-independent sources. In mammals, to date, three distinct NOS isoforms have been identified: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). The molecular structure, enzymology and pharmacology of these enzymes have been well defined, and reveal critical roles for the NOS system in a variety of important physiological processes. This review focuses on recent advances in the understanding of the interactions between NOS enzymes and pathophysiology of cardiovascular diseases (CVD) and the role of NO agonists as potential therapeutic agents in treatment of CVD.

Keywords: nitric oxide, nitric oxide synthase, endothelial dysfunction, chronic heart failure.

INTRODUCTION

Nitric oxide (NO) is a gaseous lipophilic free radical which reacts with various molecules to cause multiple biological effects and it is generated by three distinct isoforms of nitric oxide synthases (NOS), neuronal (nNOS), inducible (iNOS) and endothelial NOS (eNOS) [1-3]. The three NOS isoforms have similar enzymatic mechanisms that involve electron transfer for oxidation of the terminal guanidine nitrogen of L-arginine. These enzymes all require several cofactors for proper function, including tetrahydrobiopterin (BH₄), nicotinamide-adenine-dinucleotide phosphate (NAD-PH), flavin adenine dinucleotide, and flavin mononucleotide. Most of the effects of NO, on smooth muscle cells, platelets and cardiac myocytes, are mediated through its activation of soluble guanylate cyclase (cGMC) and amplifying the production of cyclic guanosine monophosphate (cGMP) [4, 5] but the increasing evidences indicate that NO mediates its effects also, through cGMC-independent mechanisms [5, 6]. NO reacts exclusively with other paramagnetic species, such as other radicals or metal centers, due to the presence of an unpaired electron [7]. It can react with reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot-}$) to form more active intermediate, such as peroxynitrite, in reaction that is six time faster than the dismutation of $O_2^{\cdot-}$ by superoxide dismutase (SOD) [3, 8]. Peroxynitrite directly oxidises cysteine and tryptophan, while modification of lysine, and

arginine probably occurs *via* secondary reactions with lipid hydroperoxide radicals [3, 9]. NO, unlike other intracellular molecules, is freely diffusible and influences a number of biosynthetic, metabolic, signaling and membrane transport processes [10]. NO dilates the vascular tree and inhibits platelet aggregation, thrombus formation, leukocyte adhesion and vascular proliferation [11]. Exogenous and endogenous NO [12] inhibits vascular smooth muscle cell (VSMC) proliferation [11] and migration [12]. Reduced NO bioavailability is implicated in the development of vascular diseases, although it is poorly understood whether this is a cause of, or result of endothelial dysfunction (ED) or both pathogenic events [3, 13, 14].

The existing knowledge on the role of NO in physiological and pathophysiological states has opened up a wide range of possibilities, especially in our understanding of the mechanisms of actions of drugs that modulate NO action. The high level of inducible NO is thought to be a major factor in the severe hypotension that characterizes the toxic shock syndrome. The role of NO and NOS in regulating vascular physiology, through neuro-hormonal, renal and other non-vascular pathways, as well as direct effects on arterial smooth muscle, appear to be more intricate than was originally thought. This review will focus on NOS and NO roles in endothelial dysfunction and chronic heart failure (CHF), as opposite extremes of NO bioavailability: decreased in endothelial dysfunction and increased in CHF. In addition review will discuss the recent studies of drugs and supplements that modulate NO bioavailability and their application as a potential therapeutic agent in treatment of aforementioned CVD.

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ROLE OF NO IN ENDOTHELIAL DYSFUNCTION

Most commonly, ED is characterized by an impaired NO bioavailability due to reduced production of NO by NOS or increased breakdown by ROS and it occurs early in the development of atherosclerosis [15, 16]. Cardiovascular risk factors are often associated with ED [14], which is also prognostic for occurrence of cardiovascular events. ED links CVD to pediatric chronic kidney disease because ED plays a major role in the development of CVD [17]. Endothelium-dependent vasodilatation is mediated by NO, prostacyclin, and an endothelium-derived hyperpolarising factor (EDHF), and involves small (SK) and intermediate (IK) conductance Ca^{2+} -activated K^{+} channels. Opening of SK and IK channels is associated with EDHF-type vasodilatation, but, through increased endothelial cell Ca^{2+} influx, L-arginine uptake, and decreased ROS production, it may also lead to increased NO bioavailability and endothelium-dependent vasodilatation. Therefore, SK and IK channels may be drug targets for the treatment of endothelial dysfunction in cardiovascular disease [18].

NO produced by three different isoforms of NOS widely expressed in virtually all vascular cell types is mostly produced by the eNOS in endothelial cells where it plays a crucial role in vascular tone and structure regulation [19]. It also exerts an anti-inflammatory influence, inhibits platelets adhesion and aggregation, and prevents smooth muscle cells proliferation and migration [19]. Because of this, the loss of NO production and bioactivity could explain why diverse pathological conditions such as hypercholesterolemia, hypertension, diabetes and cigarette smoking are all considered risk factors for atherosclerosis [20].

NO is released by endothelial cells mainly in response to shear stress, but also by many other molecules such as acetylcholine, bradykinin, thrombin, adenosine diphosphate (ADP), phosphodiesterase type 5 (PDE5) inhibitors and nitrovasodilators among others, leading to a relaxation of VSMC [16, 21-29]. There are also exogenous sources of NO that could influence its availability *via* nitrites, S-nitrosothiols, N-nitroso proteins and iron nitrosyl complexes [30].

The endothelium possesses all three NOS isoforms, eNOS, nNOS and under certain conditions, for example inflammation, has the capacity to express iNOS [3, 31, 32]. eNOS is only fully functional in a dimeric form, and the functional activity of the eNOS dimer is dependent on the number of bound tetrahydrobiopterin (BH4) molecules [33]. Tsutui *et al.* provided a novel concept of the diverse roles of eNOSs system mainly contributing to the endothelium-derived hyperpolarizing factor $/\text{H}_2\text{O}_2$ responses in microvessels while serving as a NO-generating system in large arteries [32].

There are many inhibitors of biological activity of NO, such as monocytes, decreased L-arginine uptake, decreased co-factors (Ca^{2+} , calmodulin, BH4), inhibition of electron flow (nicotinamide adenine dinucleotide phosphate-oxidase (NADPH), flavins), inhibition of NOS expression, inhibition of substrate binding to NOS and NO scavengers [20]. Also increased ROS concentrations (e.g. superoxide anion) reduce the amount of bioactive NO and form toxic peroxynitrite. Peroxynitrite in turn, can "uncouple" endothelial NO

synthase to become a dysfunctional superoxide-generating enzyme that contributes to vascular oxidative stress [34]. Beside them, some oxidation products of NO with ROS and thiols, such as nitrite, S-nitrosothiols and N-nitroso proteins are nowadays regarded as physiological storage pool of NO since these reactions are reversible and this can change an image of NO being "paracrine" factor [30, 35]. Among all of them, a lot of attention in recent years is focused on BH4 bioavailability and its role as an essential cofactor for optimal NOS production of NO [36]. Hence in this section we will discuss recent advancements on BH4 role and NO bioavailability in relation to ED.

BH4 is a critical cofactor for all 3 isoforms of NOS and is involved in the reduction of the heme iron of the enzyme to ultimately form an iron-oxy species that hydroxylates L-arginine to produce NO. In the absence of BH4, the NOS enzymes produce superoxide rather than NO, a situation referred to as NOS uncoupling [36, 37]. Oxidation of BH4 leads to eNOS uncoupling in a variety of other disease states including atherosclerosis, myocardial infarction, and diabetes [38-41]. Recent *in vitro* studies with purified eNOS, implicated it as an important source of vascular ROS production [41]. However, in the intact cell, BH4 depletion alone does not appear to be sufficient of an insult to trigger eNOS uncoupling. Increased levels of the BH4 oxidation product dihydrobiopterin (BH2), rather than BH4 depletion alone, is the molecular trigger for NO insufficiency and eNOS uncoupling. As such, there are three states of eNOS in regard to the biopterin cofactor: BH4-eNOS (the functional NO synthase), BH2-eNOS, and biopterin-free eNOS, both of which are uncoupled- eNOS, which have oxidase activity [41].

It is becoming increasingly clear that oxidative stress and perturbation of redox equilibrium in the endothelium, are of central importance for NOS activity and NO production [42] and that it can be seen and evaluated by redox balance of biopterin cofactors. One mechanism of BH4 oxidation is through laminar shear stress that acutely stimulates endothelial production of NO and over the long term enhances eNOS gene expression [43]. It has been shown that BH4 deficiency and NOS uncoupling likely contribute to the vascular inflammation and abnormal cytokine milieu induced by disturbed flow without affecting systemic immune cell numbers. The other mechanism of BH4 oxidation and subsequent NOS uncoupling is *via* O_2^- produced by NADPH oxidases activated through oscillatory shear stress. ROS produced by uncoupled NOS could further activate NADPH oxidases, in a feed-forward fashion and that could contribute to ROS production throughout the vessel wall. Oral BH4 supplementation prevented NOS uncoupling and improved endothelial function in the carotid exposed to disturbed flow induced by partial carotid ligation [36]. These findings highlight a pivotal role of BH4 deficiency and NOS uncoupling in atherosclerosis progression, particularly under the patterns of low and oscillatory shear flow, and indicate that modulation of vascular BH4 levels could be a therapeutic target for preventing atherosclerosis at branches and curvatures in the arterial tree. [36]. Opposite to that, studies in humans and laboratory animals have shown that the shear stress induced by physical exercise is a powerful stimulant for the release of vasorelaxing factors produced by the vascular endothelium,

such as NO and the EDHF, resulting in a decrease of arterial pressure levels [14].

There is extensive evidence that thiols potentiate eNOS activity and alleviate oxidative stress [44, 45]. NOS uncoupling induces oxidative stress and has previously been shown to occur with depletion of L-Arginine or BH4 and elevation of methylarginine levels [46-49]. It has been reported that eNOS possesses specific redox-sensitive thiols that are readily S-glutathionylated in endothelial cells and vessels with marked endothelial dysfunction and hypertension [50]. This oxidative modification switches eNOS from its classical NO synthase function to that of an NADPH-dependent oxidase generating O_2^- . Because NO and O_2^- have many opposing roles in cell signalling and vascular function [50], S-glutathionylation of eNOS will trigger profound changes in cellular and vascular function and will mediate redox-signalling under oxidative stress. Therapeutics with thiol-reducing properties can therefore now be developed and refined as potent drugs for reversing ED and ameliorating hypertension and other cardiovascular disease [51]. Therapeutically, drugs in clinical use such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin II type 1 (AT1) receptor blockers, and statins have pleiotropic actions that can improve endothelial function. Also, dietary polyphenolic antioxidants can reduce oxidative stress [34].

There is a whole relatively new area of research that investigates a beneficial role of red wine on cardiovascular system. The protective effect most likely involves the ability of red wine polyphenols (RWPs) to reduce vascular oxidative stress possibly by preventing the upregulation of NADPH oxidase and the angiotensin system [52]. Direct incubation of endothelial progenitor cell (EPC) with RW and resveratrol can modify the functions of EPC, including attenuation of senescence and promotion of EPC adhesion, migration, and tube formation. These data suggest that RW ingestion may alter the biology of EPC, and these alterations may contribute to its unique cardiovascular protective effect [53].

The availability and transport of L-arginine modulate rates of NO biosynthesis in circulating blood cells and vasculature, which provides a protective effect against cardiovascular diseases. Growing evidence shows that depression is a risk factor for the development of coronary artery disease (CAD). In depressive patients, the L-arginine-nitric oxide pathway seems to be impaired. Further clinical studies are needed to confirm the beneficial effects of L-arginine uptake on amelioration of symptoms in patients with CAD and depression [54].

Variations in the eNOS gene could have a plethora of effects on the enzyme including altered protein stability, altered post-translational processing such as acylation or phosphorylation, altered intracellular distribution or altered cofactor association: all of which could influence the enzymatic activity [3]. Among the genes that may potentially influence the onset and the progression of CVD, there are those controlling the following: renin-angiotensin-aldosterone system (RAAS), adrenergic receptors, paraoxonases, endothelin and NOS [55]. There are also recent emerging evidences that gene NOS3 polymorphism is associated with increased risk in certain CVD patient groups

as well as in patients suffering from nephropathy and diabetes. For example, The 894T and -786C alleles of the NOS3 gene are significantly associated with both hypertension and CVD in renal allograft recipients [56]. In addition, The G894T polymorphism of the eNOS gene is associated with severity of renal disease and the presence of the 894T allele aggravated renal damage and increased the incidence of CVD in Tunisian CRD patients [57]. Gonzales *et al.* showed that NOS2A or NOS3 gene polymorphisms do not infer a direct risk for CV events in rheumatoid arthritis. However, some interactions between NOS gene polymorphisms and HLA-DRB1 alleles confer an increased risk of developing CV events in patients with rheumatoid arthritis [58]. NOS3-gene may be involved in the development of diabetic nephropathy in patients with type 1 diabetes and can be predictive of CVD during follow-up [59]. On the other hand, the need for large-scale genetic association studies using tagging polymorphisms is warranted to confirm or refute a role of the NOS3 gene in coronary heart disease [60].

NO IN CHF-EFFECTS OF CARVEDILOL

CHF is a complex clinical syndrome that can result from any structural or functional cardiac or non cardiac disorder that impairs the ability of the heart to respond to physiological demands for increased cardiac output [61].

The role of NO in CHF is complex. On the one hand, lack of NO is leading to ED with its detrimental consequences including impaired tissue perfusion, myocardial ischaemia, and vascular remodeling [35]. On the other hand, higher concentrations of NO, which have been observed in the failing myocardium, may cause the loss of myocytes and inhibit myocyte contractility [62, 63].

High quantities of NO released during septicemia result in cardiovascular collapse and eventual death. Previous studies have also reported increased myocardial iNOS expression and activity in CHF [64, 65]. Indeed, because high concentrations of NO attenuate myocyte contraction and catecholamine responses [66, 67], one proposed mechanism of myocardial dysfunction in CHF is excessive NO production secondary to increased inflammatory cytokines. In support of this concept, studies have shown that NOS blockade improves myocardial beta-adrenergic responsiveness [65, 68].

Recent investigations have shown that in failing myocardium, chronic beta-adrenergic blockade improves myocardial function and left ventricular (LV) remodeling, although the cellular mechanisms responsible for these salutary effects have not been fully defined [69]. In addition, for example, in dogs with pacing-induced cardiac failure [70] a reduction in O_2 consumption has been observed, and consequently, in coronary flow, suggesting a down-regulation of energy metabolism. It has been also seen that in dogs with heart failure (HF), selective iNOS inhibition with S-methyl-isothioureia increased LV contractility and oxygen consumption at rest and during exercise, indicating that, unlike what is observed in normal hearts, in failing hearts NO in excess is mainly produced by iNOS rather than by eNOS [71]. The increase in coronary blood flow after inhibition of the release of NO is clearly due to the prevalence of the effect mediated by the increased oxygen

consumption on the vessels that would be otherwise counteracted by a reduced concentration of NO in the vascular wall. From this [72] investigation it seems that the reduction in O₂ consumption, which may take place in CHF, depends on the increased availability of NO [73-75]. It has been shown that NO has a negative effect on β -adrenergic response of ventricular myocardium that appears to be enhanced in failing myocardium. Moreover, β -adrenergic stimulation by isoproterenol increases NO release [73-75] and can amplify its depressant modulation. It may then be argued that in HF the effect of an increased β -adrenergic activation on myocardium is attenuated by a sort of negative functional feedback. Since a long lasting β -adrenergic activation is a maladaptive phenomenon, this feedback can play a protective role against the progression of CHF [73-75].

Carvedilol, a nonselective beta blocker with antioxidant and α -adrenergic receptor blocking ('alpha-blocking') activities, is one of the most effective beta blockers in reducing ventricular tachyarrhythmias and mortality in individuals with HF [76-80]. The reports on the effect of carvedilol on NO production are scarce yet controversial. Kurosaki *et al.* reported that carvedilol stimulated the expression of iNOS in cardiac myocytes [81]. In addition, one *in vivo* study using rats indicated that the drug decreased arterial pressure whilst it increased NO production [82]. On the other side, Pecivova *et al.* reported that carvedilol does not affect the expression of iNOS by macrophages [83] and Yoshioka *et al.*, (2000) demonstrated a novel effect of carvedilol as a NO quenching agent. Their results indicate that carvedilol directly interacts with NO in a cell-free system [84].

In the clinical setting, the effects of carvedilol on NO may be diverse depending on the local concentrations of free radicals and NO. The proposed antioxidant mechanisms of carvedilol include: (1) direct interaction with oxygen radicals; (2) prevention of the depletion of intracellular antioxidants; (3) attenuation of iron-mediated free radical formation [85, 86].

The antioxidant and alpha-blocking activities of carvedilol have been suggested to contribute to its beneficial effects, but the benefits of antioxidants and alpha blockers have not been corroborated by clinical studies [87, 88]. Zhou *et al.* found that carvedilol is the only beta blocker tested that can effectively suppress arrhythmogenic store overload-induced Ca²⁺ release that can lead to triggered arrhythmias and sudden death [76, 89]. Carvedilol did so by directly altering the function of channel ryanodine receptors 2, independent of its beta- or alpha-blocking or antioxidant activities [76].

Regarding other antioxidants and their potential role in ameliorating increased content of NO and ROS in CHF, no trials have been identified on the effect of omega-3 capsules on morbidity in patients with CHF [90]. One trial of 56 patients with advanced heart failure indicated that supplementation with vitamin E did not result in any significant improvements in prognostic or functional indexes of heart failure or in quality of life [91]. On the other hand Horning *et al.* were the first to show that acute intra-arterial administration or oral intake of vitamin C over a period of 4

weeks significantly improved flow-mediated dilatation by about 50% in patients with CHF [92].

In summary, the overexpression of iNOS and the increased production of NO seem to characterize the CHF. This is accompanied by an increased ROS production, which may lead to ONOO⁻ (peroxynitrite) formation. NO oxide has a negative impact on β -adrenergic response of ventricular myocardium that is enhanced in failing myocardium [85].

CONCLUSION

Increasing knowledge of the role of iNOS in heart has stimulated efforts to target NO pathway pharmacologically. On the basis of survey presented in this review, we can say that NO is a very important "messenger molecule" so far as its spectrum of actions is concerned. This molecule plays a vital role in a wide variety of pathophysiological and biochemical reactions. In summary, NO has been acknowledged as a critically important mediator in pathophysiology of different cardiac diseases. NO has been identified as one of the key targets for novel therapeutic interventions to minimize irreversible tissue damage associated with CVD. A safer technology to regulate *in vivo* synthesis of NO by generic manipulation would be a welcome work.

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CONFLICT OF INTEREST

None declared.

REFERENCES

- [1] Cohen, R. A. The potential clinical impact of 20 years of nitric oxide research. *Am. J. Physiol.*, **1999**, 276, H1404-1407.
- [2] Grisham, M. B.; Jourdain, D.; Wink, D. A. Nitric oxide. I. Physiological chemistry of nitric oxide and its metabolites: implications in inflammation. *Am. J. Physiol.*, **1999**, 276, G315-321.
- [3] Naseem, K. M. The role of nitric oxide in cardiovascular diseases. *Mol. Aspect. Med.*, **2005**, 26, 33-65.
- [4] Mayer, B.; Schrammel, A.; Klatt, P.; Koesling, D.; Schmidt, K. Peroxynitrite-induced accumulation of cyclic GMP in endothelial cells and stimulation of purified soluble guanylyl cyclase. Dependence on glutathione and possible role of S-nitrosation. *J. Biol. Chem.*, **1995**, 270, 17355-17360.
- [5] Bian, K.; Doursout, M. F.; Murad, F. Vascular system: role of nitric oxide in cardiovascular diseases. *J. Clin. Hypertens. (Greenwich)*, **2008**, 10, 304-310.
- [6] Hobbs, A. J. Soluble guanylate cyclase: the forgotten sibling. *Trends Pharmacol. Sci.*, **1997**, 18, 484-491.
- [7] Radi, R. Kinetic analysis of reactivity of peroxynitrite with biomolecules. *Methods Enzymol.*, **1996**, 269, 354-366.
- [8] Beckman, J. S.; Koppenol, W. H. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am. J. Physiol.*, **1996**, 271, C1424-1437.
- [9] Mungrue, I. N.; Bredt, D. S.; Stewart, D. J.; Husain, M. From molecules to mammals: what's NOS got to do with it? *Acta Physiol. Scand.*, **2003**, 179, 123-135.

- [10] Mayer, B.; Hemmens, B. Biosynthesis and action of nitric oxide in mammalian cells. *Trends Biochem. Sci.*, **1997**, *22*, 477-481.
- [11] Sarkar, R.; Webb, R. C. Does nitric oxide regulate smooth muscle cell proliferation? A critical appraisal. *J. Vasc. Res.*, **1998**, *35*, 135-142.
- [12] Sarkar, R.; Meinberg, E. G.; Stanley, J. C.; Gordon, D.; Webb, R. C. Nitric oxide reversibly inhibits the migration of cultured vascular smooth muscle cells. *Circ. Res.*, **1996**, *78*, 225-230.
- [13] Napoli, C.; Ignarro, L. J. Nitric oxide and pathogenic mechanisms involved in the development of vascular diseases. *Arch. Pharm. Res.*, **2009**, *32*, 1103-1108.
- [14] Zago, A. S.; Zanesco, A. Nitric oxide, cardiovascular disease and physical exercise. *Arq. Bras. Cardiol.*, **2006**, *87*, e264-270.
- [15] Lüscher, T. F.; Vanhoutte, P. M., *The Endothelium: Modulator of Cardiovascular Function*. Boca Raton: CRC Press: **1990**.
- [16] Flammer, A. J.; Luscher, T. F. Three decades of endothelium research: from the detection of nitric oxide to the everyday implementation of endothelial function measurements in cardiovascular diseases. *Swiss Med. Wkly.*, **2010**, *140*, w13122.
- [17] Tain, Y. L. Endothelial dysfunction links cardiovascular disease to pediatric chronic kidney disease: the role of nitric oxide deficiency. *Acta Paediatr. Taiwan*, **2007**, *48*, 246-250.
- [18] Dalsgaard, T.; Kroigaard, C.; Simonsen, U. Calcium-activated potassium channels - a therapeutic target for modulating nitric oxide in cardiovascular disease? *Expert Opin. Ther. Targets*, **2010**, *14*, 825-837.
- [19] Desjardins, F.; Balligand, J. L. Nitric oxide-dependent endothelial function and cardiovascular disease. *Acta Clin. Belg.*, **2006**, *61*, 326-334.
- [20] Yetik-Anacak, G.; Catravas, J. D. Nitric oxide and the endothelium: history and impact on cardiovascular disease. *Vasc. Pharmacol.*, **2006**, *45*, 268-276.
- [21] Rubanyi, G. M.; Romero, J. C.; Vanhoutte, P. M. Flow-induced release of endothelium-derived relaxing factor. *Am. J. Physiol.*, **1986**, *250*, H1145-1149.
- [22] Anderson, E. A.; Mark, A. L. Flow-mediated and reflex changes in large peripheral artery tone in humans. *Circulation*, **1989**, *79*, 93-100.
- [23] Palmer, R. M.; Ashton, D. S.; Moncada, S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature*, **1988**, *333*, 664-666.
- [24] Palmer, R. M.; Rees, D. D.; Ashton, D. S.; Moncada, S. L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. *Biochem. Biophys. Res. Commun.*, **1988**, *153*, 1251-1256.
- [25] Vallance, P.; Collier, J.; Moncada, S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet*, **1989**, *2*, 997-1000.
- [26] Joannides, R.; Richard, V.; Haefeli, W. E.; Linder, L.; Luscher, T. F.; Thuillez, C. Role of basal and stimulated release of nitric oxide in the regulation of radial artery caliber in humans. *Hypertension*, **1995**, *26*, 327-331.
- [27] Joannides, R.; Haefeli, W. E.; Linder, L.; Richard, V.; Bakali, E. H.; Thuillez, C.; Luscher, T. F. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries *in vivo*. *Circulation*, **1995**, *91*, 1314-1319.
- [28] Stamler, J. S.; Loh, E.; Roddy, M. A.; Currie, K. E.; Creager, M. A. Nitric oxide regulates basal systemic and pulmonary vascular resistance in healthy humans. *Circulation*, **1994**, *89*, 2035-2040.
- [29] Boerrigter, G.; Burnett, J. C., Jr. Nitric oxide-independent stimulation of soluble guanylate cyclase with BAY 41-2272 in cardiovascular disease. *Cardiovasc. Drug Rev.*, **2007**, *25*, 30-45.
- [30] Lundberg, J. O. Nitric oxide metabolites and cardiovascular disease markers, mediators, or both? *J. Am. Coll. Cardiol.*, **2006**, *47*, 580-581.
- [31] Kibbe, M.; Billiar, T.; Tzeng, E. Inducible nitric oxide synthase and vascular injury. *Cardiovasc. Res.*, **1999**, *43*, 650-657.
- [32] Tsutsui, M.; Shimokawa, H.; Otsuji, Y.; Yanagihara, N. Pathophysiological relevance of NO signaling in the cardiovascular system: novel insight from mice lacking all NO synthases. *Pharmacol. Ther.*, **2010**, *128*, 499-508.
- [33] Miyazaki-Akita, A.; Hayashi, T.; Ding, Q. F.; Shiraishi, H.; Nomura, T.; Hattori, Y.; Iguchi, A. 17beta-estradiol antagonizes the down-regulation of endothelial nitric-oxide synthase and GTP cyclohydrolase I by high glucose: relevance to postmenopausal diabetic cardiovascular disease. *J. Pharmacol. Exp. Ther.*, **2007**, *320*, 591-598.
- [34] Forstermann, U. Nitric oxide and oxidative stress in vascular disease. *Pflugers Arch.*, **2010**, *459*, 923-939.
- [35] Giles, T. D. Aspects of nitric oxide in health and disease: a focus on hypertension and cardiovascular disease. *J. Clin. Hypertens. (Greenwich)*, **2006**, *8*, 2-16.
- [36] Li, L.; Chen, W.; Rezvan, A.; Jo, H.; Harrison, D. G. Tetrahydrobiopterin deficiency and nitric oxide synthase uncoupling contribute to atherosclerosis induced by disturbed flow. *Arterioscler. Thromb. Vasc. Biol.*, **2011**, *31*, 1547-1554.
- [37] Vasquez-Vivar, J.; Kalyanaram, B.; Martasek, P. The role of tetrahydrobiopterin in superoxide generation from eNOS: enzymology and physiological implications. *Free Radic. Res.*, **2003**, *37*, 121-127.
- [38] Vasquez-Vivar, J.; Martasek, P.; Whitsett, J.; Joseph, J.; Kalyanaram, B. The ratio between tetrahydrobiopterin and oxidized tetrahydrobiopterin analogues controls superoxide release from endothelial nitric oxide synthase: an EPR spin trapping study. *Biochem. J.*, **2002**, *362*, 733-739.
- [39] Cosentino, F.; Hurlimann, D.; Delli Gatti, C.; Chenevard, R.; Blau, N.; Alp, N. J.; Channon, K. M.; Eto, M.; Lerch, P.; Enseleit, F.; Ruschitzka, F.; Volpe, M.; Luscher, T. F.; Noll, G. Chronic treatment with tetrahydrobiopterin reverses endothelial dysfunction and oxidative stress in hypercholesterolaemia. *Heart*, **2008**, *94*, 487-492.
- [40] Alp, N. J.; McAteer, M. A.; Khoo, J.; Choudhury, R. P.; Channon, K. M. Increased endothelial tetrahydrobiopterin synthesis by targeted transgenic GTP-cyclohydrolase I overexpression reduces endothelial dysfunction and atherosclerosis in ApoE-knockout mice. *Arterioscler. Thromb. Vasc. Biol.*, **2004**, *24*, 445-450.
- [41] Karuppiah, K.; Druhan, L. J.; Chen, C. A.; Smith, T.; Zweier, J. L.; Sessa, W. C.; Cardounel, A. J. Suppression of eNOS-derived superoxide by caveolin-1: a biopterin-dependent mechanism. *Am. J. Physiol. Heart Circ. Physiol.*, **2011**, *301*, H903-911.
- [42] Elahi, M. M.; Naseem, K. M.; Matata, B. M. Nitric oxide in blood. The nitrosative-oxidative disequilibrium hypothesis on the pathogenesis of cardiovascular disease. *FEBS J.*, **2007**, *274*, 906-923.
- [43] Kawashima, S.; Yokoyama, M. Dysfunction of endothelial nitric oxide synthase and atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.*, **2004**, *24*, 998-1005.
- [44] Kugiyama, K.; Ohgushi, M.; Motoyama, T.; Hirashima, O.; Soejima, H.; Misumi, K.; Yoshimura, M.; Ogawa, H.; Sugiyama, S.; Yasue, H. Intracoronary infusion of reduced glutathione improves endothelial vasomotor response to acetylcholine in human coronary circulation. *Circulation*, **1998**, *97*, 2299-2301.
- [45] Vita, J. A.; Frei, B.; Holbrook, M.; Gokce, N.; Leaf, C.; Keane, J. F., Jr. L-2-Oxothiazolidine-4-carboxylic acid reverses endothelial dysfunction in patients with coronary artery disease. *J. Clin. Invest.*, **1998**, *101*, 1408-1414.
- [46] Druhan, L. J.; Forbes, S. P.; Pope, A. J.; Chen, C. A.; Zweier, J. L.; Cardounel, A. J. Regulation of eNOS-derived superoxide by endogenous methylarginines. *Biochemistry*, **2008**, *47*, 7256-7263.
- [47] Cardounel, A. J.; Xia, Y.; Zweier, J. L. Endogenous methylarginines modulate superoxide as well as nitric oxide generation from neuronal nitric-oxide synthase: differences in the effects of monomethyl- and dimethylarginines in the presence and absence of tetrahydrobiopterin. *J. Biol. Chem.*, **2005**, *280*, 7540-7549.
- [48] Xia, Y.; Dawson, V. L.; Dawson, T. M.; Snyder, S. H.; Zweier, J. L. Nitric oxide synthase generates superoxide and nitric oxide in arginine-depleted cells leading to peroxynitrite-mediated cellular injury. *Proc. Natl. Acad. Sci. U S A.*, **1996**, *93*, 6770-6774.
- [49] Xia, Y.; Tsai, A. L.; Berka, V.; Zweier, J. L. Superoxide generation from endothelial nitric-oxide synthase. A Ca²⁺/calmodulin-dependent and tetrahydrobiopterin regulatory process. *J. Biol. Chem.*, **1998**, *273*, 25804-25808.

- [50] Pryor, W. A.; Houk, K. N.; Foote, C. S.; Fukuto, J. M.; Ignarro, L. J.; Squadrito, G. L.; Davies, K. J. Free radical biology and medicine: it's a gas, man! *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **2006**, *291*, R491-511.
- [51] Chen, C. A.; Wang, T. Y.; Varadharaj, S.; Reyes, L. A.; Hemann, C.; Talukder, M. A.; Chen, Y. R.; Druhan, L. J.; Zweier, J. L. S-glutathionylation uncouples eNOS and regulates its cellular and vascular function. *Nature*, **2010**, *468*, 1115-1118.
- [52] Dal-Ros, S.; Zoll, J.; Lang, A. L.; Auger, C.; Keller, N.; Bronner, C.; Geny, B.; Schini-Kerth, V. B. Chronic intake of red wine polyphenols by young rats prevents aging-induced endothelial dysfunction and decline in physical performance: role of NADPH oxidase. *Biochem. Biophys. Res. Commun.*, **2011**, *404*, 743-749.
- [53] Huang, P. H.; Chen, Y. H.; Tsai, H. Y.; Chen, J. S.; Wu, T. C.; Lin, F. Y.; Sata, M.; Chen, J. W.; Lin, S. J. Intake of red wine increases the number and functional capacity of circulating endothelial progenitor cells by enhancing nitric oxide bioavailability. *Arterioscler. Thromb. Vasc. Biol.*, **2010**, *30*, 869-877.
- [54] Pinto, V. L.; Brunini, T. M.; Ferraz, M. R.; Okinga, A.; Mendes-Ribeiro, A. C. Depression and cardiovascular disease: role of nitric oxide. *Cardiovasc. Hematol. Agents Med. Chem.*, **2008**, *6*, 142-149.
- [55] Gluba, A.; Banach, M.; Mikhailidis, D. P.; Rysz, J. Genetic determinants of cardiovascular disease: the renin-angiotensin-aldosterone system, paraoxonases, endothelin-1, nitric oxide synthase and adrenergic receptors. *In vivo*, **2009**, *23*, 797-812.
- [56] Bhandary, U. V.; Tse, W.; Yang, B.; Knowles, M. R.; Demaine, A. G. Endothelial nitric oxide synthase polymorphisms are associated with hypertension and cardiovascular disease in renal transplantation. *Nephrology (Carlton)*, **2008**, *13*, 348-355.
- [57] Kerkeni, M.; Letaief, A.; Achour, A.; Miled, A.; Trivin, F.; Maaroufi, K. Endothelial nitric oxide synthetase, methylenetetrahydrofolate reductase polymorphisms, and cardiovascular complications in Tunisian patients with nondiabetic renal disease. *Clin. Biochem.*, **2009**, *42*, 958-964.
- [58] Gonzalez-Gay, M. A.; Llorca, J.; Palomino-Morales, R.; Gomez-Acebo, I.; Gonzalez-Juanatey, C.; Martin, J. Influence of nitric oxide synthase gene polymorphisms on the risk of cardiovascular events in rheumatoid arthritis. *Clin. Exp. Rheumatol.*, **2009**, *27*, 116-119.
- [59] Mollsten, A.; Lajer, M.; Jorsal, A.; Tarnow, L. The endothelial nitric oxide synthase gene and risk of diabetic nephropathy and development of cardiovascular disease in type 1 diabetes. *Mol. Genet. Metab.*, **2009**, *97*, 80-84.
- [60] Casas, J. P.; Cavalleri, G. L.; Bautista, L. E.; Smeeth, L.; Humphries, S. E.; Hingorani, A. D. Endothelial nitric oxide synthase gene polymorphisms and cardiovascular disease: a HuGE review. *Am. J. Epidemiol.*, **2006**, *164*, 921-935.
- [61] Network, S. I. G., Management of chronic heart failure: a national clinical guideline. Scottish Intercollegiate Guidelines Network: 2007; Vol. 95, pp 1-49.
- [62] Drexler, H.; Hornig, B. Endothelial dysfunction in human disease. *J. Mol. Cell. Cardiol.*, **1999**, *31*, 51-60.
- [63] Anker, S. D.; von Haehling, S. Inflammatory mediators in chronic heart failure: an overview. *Heart*, **2004**, *90*, 464-470.
- [64] Haywood, G. A.; Tsao, P. S.; von der Leyen, H. E.; Mann, M. J.; Keeling, P. J.; Trindade, P. T.; Lewis, N. P.; Byrne, C. D.; Rickenbacher, P. R.; Bishopric, N. H.; Cooke, J. P.; McKenna, W. J.; Fowler, M. B. Expression of inducible nitric oxide synthase in human heart failure. *Circulation*, **1996**, *93*, 1087-1094.
- [65] Yamamoto, S.; Tsutsui, H.; Tagawa, H.; Saito, K.; Takahashi, M.; Tada, H.; Yamamoto, M.; Katoh, M.; Egashira, K.; Takeshita, A. Role of myocyte nitric oxide in beta-adrenergic hyporesponsiveness in heart failure. *Circulation*, **1997**, *95*, 1111-1114.
- [66] Balligand, J. L.; Kelly, R. A.; Marsden, P. A.; Smith, T. W.; Michel, T. Control of cardiac muscle cell function by an endogenous nitric oxide signaling system. *Proc. Natl. Acad. Sci. U. S. A.*, **1993**, *90*, 347-351.
- [67] Brady, A. J.; Warren, J. B.; Poole-Wilson, P. A.; Williams, T. J.; Harding, S. E. Nitric oxide attenuates cardiac myocyte contraction. *Am. J. Physiol.*, **1993**, *265*, H176-182.
- [68] Hare, J. M.; Loh, E.; Creager, M. A.; Colucci, W. S. Nitric oxide inhibits the positive inotropic response to beta-adrenergic stimulation in humans with left ventricular dysfunction. *Circulation*, **1995**, *92*, 2198-2203.
- [69] Bristow, M. R. Mechanism of action of beta-blocking agents in heart failure. *Am. J. Cardiol.*, **1997**, *80*, 26L-40L.
- [70] Traverse, J. H.; Chen, Y.; Hou, M.; Bache, R. J. Inhibition of NO production increases myocardial blood flow and oxygen consumption in congestive heart failure. *Am. J. Physiol. Heart Circ. Physiol.*, **2002**, *282*, H2278-2283.
- [71] Chen, Y.; Traverse, J. H.; Du, R.; Hou, M.; Bache, R. J. Nitric oxide modulates myocardial oxygen consumption in the failing heart. *Circulation*, **2002**, *106*, 273-279.
- [72] Rastaldo, R.; Pagliaro, P.; Cappello, S.; Penna, C.; Mancardi, D.; Westerhof, N.; Losano, G. Nitric oxide and cardiac function. *Life Sci.*, **2007**, *81*, 779-793.
- [73] Isenovic, E.; Muniyappa, R.; Milivojevic, N.; Rao, Y.; Sowers, J. R. Role of PI3-kinase in isoproterenol and IGF-1 induced eNOS activity. *Biochem. Biophys. Res. Commun.*, **2001**, *285*, 954-958.
- [74] Isenovic, E.; Walsh, M. F.; Muniyappa, R.; Bard, M.; Diglio, C. A.; Sowers, J. R. Phosphatidylinositol 3-kinase may mediate isoproterenol-induced vascular relaxation in part through nitric oxide production. *Metabolism*, **2002**, *51*, 380-386.
- [75] Kanai, A. J.; Mesaros, S.; Finkel, M. S.; Oddis, C. V.; Birder, L. A.; Malinski, T. Beta-adrenergic regulation of constitutive nitric oxide synthase in cardiac myocytes. *Am. J. Physiol.*, **1997**, *273*, C1371-1377.
- [76] Zhou, Q.; Xiao, J.; Jiang, D.; Wang, R.; Vembaiyan, K.; Wang, A.; Smith, C. D.; Xie, C.; Chen, W.; Zhang, J.; Tian, X.; Jones, P. P.; Zhong, X.; Guo, A.; Chen, H.; Zhang, L.; Zhu, W.; Yang, D.; Li, X.; Chen, J.; Gillis, A. M.; Duff, H. J.; Cheng, H.; Feldman, A. M.; Song, L. S.; Fill, M.; Back, T. G.; Chen, S. R. Carvedilol and its new analogs suppress arrhythmogenic store overload-induced Ca(2+) release. *Nat. Med.*, **2011**, *17*, 1003-1009.
- [77] Poole-Wilson, P. A.; Swedberg, K.; Cleland, J. G.; Di Lenarda, A.; Hanrath, P.; Komajda, M.; Lubsen, J.; Lutiger, B.; Metra, M.; Remme, W. J.; Torp-Pedersen, C.; Scherhag, A.; Skene, A. Comparison of carvedilol and metoprolol on clinical outcomes in patients with chronic heart failure in the Carvedilol or Metoprolol European Trial (COMET): randomised controlled trial. *Lancet*, **2003**, *362*, 7-13.
- [78] Stroe, A. F.; Gheorghide, M. Carvedilol: beta-blockade and beyond. *Rev. Cardiovasc. Med.*, **2004**, *5* (Suppl 1), S18-27.
- [79] Greenberg, B. Nonselective versus selective beta-blockers in the management of chronic heart failure: clinical implications of the carvedilol or Metoprolol European Trial. *Rev. Cardiovasc. Med.*, **2004**, *5* (Suppl 1), S10-17.
- [80] Remme, W. J. Which beta-blocker is most effective in heart failure? *Cardiovasc. Drugs Ther.*, **2010**, *24*, 351-358.
- [81] Kurosaki, K.; Ikeda, U.; Maeda, Y.; Shimada, K. Carvedilol stimulates nitric oxide synthesis in rat cardiac myocytes. *J. Mol. Cell. Cardiol.*, **2000**, *32*, 333-339.
- [82] Afonso, R. A.; Patarrao, R. S.; Macedo, M. P.; Carmo, M. M. Carvedilol action is dependent on endogenous production of nitric oxide. *Am. J. Hypertens.*, **2006**, *19*, 419-425.
- [83] Pecivova, J.; Macickova, T.; Lojek, A.; Gallova, L.; Ciz, M.; Nosal, R.; Holomanova, D. *In vitro* effect of carvedilol on professional phagocytes. *Pharmacology*, **2007**, *79*, 86-92.
- [84] Yoshioka, T.; Iwamoto, N.; Tsukahara, F.; Irie, K.; Urakawa, I.; Muraki, T. Anti-NO action of carvedilol in cell-free system and in vascular endothelial cells. *Br. J. Pharmacol.*, **2000**, *129*, 1530-1535.
- [85] Yue, T. L.; Cheng, H. Y.; Lysko, P. G.; McKenna, P. J.; Feuerstein, R.; Gu, J. L.; Lysko, K. A.; Davis, L. L.; Feuerstein, G. Carvedilol, a new vasodilator and beta adrenoceptor antagonist, is an antioxidant and free radical scavenger. *J. Pharmacol. Exp. Ther.*, **1992**, *263*, 92-98.
- [86] Tadolini, B.; Franconi, F. Carvedilol inhibition of lipid peroxidation. A new antioxidative mechanism. *Free Radic. Res.*, **1998**, *29*, 377-387.
- [87] Kukin, M. L.; Kalman, J.; Mannino, M.; Freudenberger, R.; Buchholz, C.; Ocampo, O. Combined alpha-beta blockade

- (doxazosin plus metoprolol) compared with beta blockade alone in chronic congestive heart failure. *Am. J. Cardiol.*, **1996**, *77*, 486-491.
- [88] Thomson, M. J.; Puntmann, V.; Kaski, J. C. Atherosclerosis and oxidant stress: the end of the road for antioxidant vitamin treatment? *Cardiovasc. Drugs Ther.*, **2007**, *21*, 195-210.
- [89] Xie, L. H.; Weiss, J. N. Arrhythmogenic consequences of intracellular calcium waves. *Am. J. Physiol. Heart Circ. Physiol.*, **2009**, *297*, H997-H1002.
- [90] Network, S. I. G. Risk estimation and the prevention of cardiovascular disease. Edinburgh, 2007.
- [91] Keith, M. E.; Jeejeebhoy, K. N.; Langer, A.; Kurian, R.; Barr, A.; O'Kelly, B.; Sole, M. J. A controlled clinical trial of vitamin E supplementation in patients with congestive heart failure. *Am. J. Clin. Nutr.*, **2001**, *73*, 219-224.
- [92] Hornig, B.; Arakawa, N.; Kohler, C.; Drexler, H. Vitamin C improves endothelial function of conduit arteries in patients with chronic heart failure. *Circulation*, **1998**, *97*, 363-368.

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