ANTIOXIDANTS & REDOX SIGNALING
Volume 10, Number 9, 2008

DOI: 10.1089/ars.2007.2013

Comprehensive Invited Review

Endothelial Dysfunction: From Molecular Mechanisms to Measurement, Clinical Implications, and Therapeutic Opportunities

Michelle Le Brocq,[1] Stephen J. Leslie,[1] Philip Milliken,[2] and Ian L. Megson[1]

Reviewing Editors: Frank Faraci, Pin-Lan Li, Subramaniam Pennathur, Chandan K. Sen, and Eric Thorin

[1]Health Faculty, UHI Millennium Institute, Inverness; and [2]Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, Scotland.

Abstract

Endothelial dysfunction has been implicated as a key factor in the development of a wide range of cardiovascular diseases, but its definition and mechanisms vary greatly between different disease processes. This review combines evidence from cell-culture experiments, *in vitro* and *in vivo* animal models, and clinical studies to identify the variety of mechanisms involved in endothelial dysfunction in its broadest sense. Several prominent disease states, including hypertension, heart failure, and atherosclerosis, are used to illustrate the different manifestations of endothelial dysfunction and to establish its clinical implications in the context of the range of mechanisms involved in its development. The size of the literature relating to this subject precludes a comprehensive survey; this review aims to cover the key elements of endothelial dysfunction in cardiovascular disease and to highlight the importance of the process across many different conditions. *Antioxid. Redox Signal*. 10, 1631–1673.

I. Introduction

The endothelium is a monolayer of cells derived from the embryonic mesoderm that form a continuous layer on the intimal surface of the entire cardiovascular system, including the arteries, veins, and chambers of the heart (endocardium); the capillary walls consist solely of endothelial cells. The endothelium was originally considered to be simply a passive interface between the blood and tissues, but it transpired that it performs a wide range of complex and wide-ranging tasks. At the microvascular level, the endothelium is central to control of vascular permeability, exerting regulatory control over transcellular, intercellular, and paracellular diffusion in response to environmental and molecular signals [reviewed in (438)]. Furthermore, the endothelium emerged to be not simply a nonadhesive barrier between the blood and prothrombotic collagen in the underlying basement membrane, but rather a cell layer that actively prevents thrombosis through expression of anticoagulants such as heparan sulfate (with properties similar to those of heparin) on its surface (90), together with enzymes that destroy, for example, circulating ADP (269). Given these properties, it is important for the endothelium to undergo rapid repair when damaged and for apoptotic cells to be quickly replaced by circulating endothelial progenitor cells (EPCs), which are also central to angiogenesis throughout our lifespan [reviewed in (434)].

However, it was a series of discoveries from the mid-1970s onward that radically changed our perception of endothelial cell function. Now, the endothelium is recognized to be a highly complex "organ" that responds to physical and chemical stimuli to generate a wide range of organic and inorganic messenger molecules that are capable of influencing the physiology of the surrounding tissue, particularly with respect to blood flow. Furthermore, the endothelium is also responsive to inflammatory activation, triggering expression of receptors and adhesion molecules, which have a quite different impact on the pathophysiology of affected tissue.

Endothelial dysfunction is a widely used term to describe any form of abnormal activity of the endothelium, encom-

passing both dysfunctional production of messenger molecules and expression of proinflammatory adhesion molecules. Dysfunction is deleterious and is implicated as a key factor in the initiation and progression of the atherogenic process that underlies coronary artery disease, peripheral ischemia, and some forms of stroke, although its role in plaque rupture is less clear. Dysfunction has also been implicated in the progression of other cardiovascular conditions, including hypertension (337) and heart failure (235,262). The literature on the topic is enormous, and this review, although lengthy, provides only a glimpse of a highly complex field.

On account of the broad range of specific definitions of endothelial dysfunction, it is essential first to categorize the different mechanisms and measures that are related to dysfunction, although it is important to note that many, if not all, of the processes described separately here interact with each other *in vivo*. In many cases, only one facet of endothelial dysfunction is investigated in any particular study, but it is more than likely that other forms also are present.

II. The Endothelium-Stimulation Processes

A wide range of processes and mediators are now known to stimulate the endothelium to produce an array of factors that mediate local vascular relaxation, contraction, platelet function, and fibrinolysis. For the purposes of clarity, we have distinguished endothelial *stimulation* (leading to increased release of vasoactive agents) from *activation* (resulting in expression of adhesion molecules and progression to a proinflammatory state). In the literature, however, these two terms are used interchangeably.

A. Physicochemical stimuli

Shear stress–the lateral force exerted on the endothelial cells by the passage of a semiviscous fluid over them–is a particularly important physical stimulus for endothelium-dependent vascular relaxation (339, 427), and it is now well established that areas of the vasculature that experience unusual shear stress are particularly vulnerable to endothelial dysfunction (402). This is particularly true in regions where blood flow is disturbed (*i.e.*, not laminar), including at bifurcations and branch points, in tortuous or curved vessels (70), and in coronary arteries (388, 389), where heart movements during the cardiac cycle contribute to unusual flow patterns. Whereas much of the work surrounding flow centers on the stimulation of the endothelium to release relaxing factors, it is also worth noting that flow affects the antioxidant systems of endothelial cells through induction of the antioxidant response element (ARE) (69). Areas that experience disturbed or turbulent flow are subject to low shear stress, leading to a failure in mechanoreceptor-mediated release of intracellular calcium, together with depression of a range of phosphorylation pathways, including AKt and protein kinases (PK) C and G, that result in reduced generation of endothelium-derived relaxing factors (31) (Fig. 1). Long-term deprivation of endothelial cells of shear stress exacerbates the issue through downregulation of critical enzymes involved in generating relaxing factors (*e.g.*, nitric oxide; NO) that are deemed to be protective, while simultaneously upregulating endogenous constrictors and proatherogenic agents (*e.g.*, endothelin-1), which promote vascular disease.

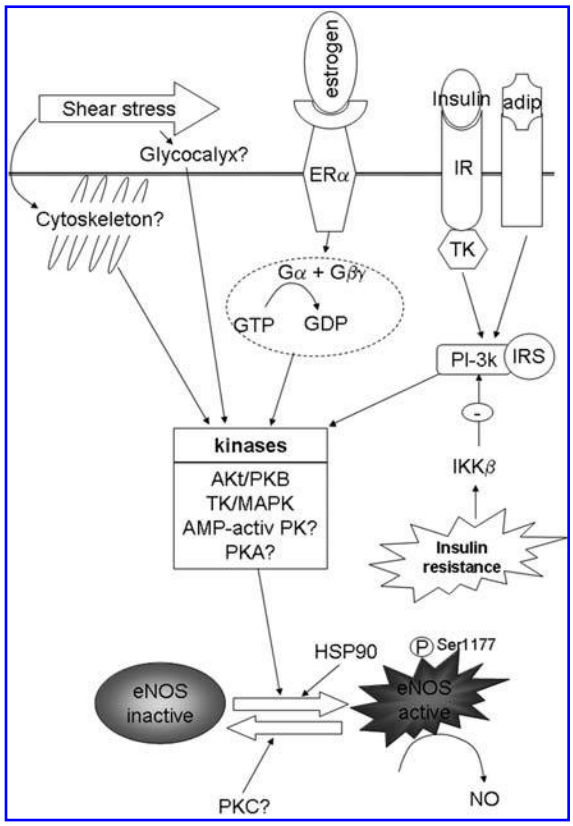

FIG. 1. Kinases and the Akt pathway: stimulators and impact of insulin resistance.

However, it is also likely that areas that experience very high shear forces are subject to an erosive effect on the endothelial cells, whereby dysfunction is precipitated through physical loss of cells (11, 116); prior apoptosis of the endothelial cells might be a prerequisite for erosion (101).

It is important to recognize that other physical stimulators of the endothelium exist besides shear stress. Wall stretch also contributes to basal endothelial activity (55), whereas some studies suggest that the level of oxygenation of the surrounding tissue is important in determining the level of local endothelium-mediated vasodilatation (211). Such a mechanism of oxygen sensing and local response makes physiologic sense, but a great deal of conflicting evidence exists as to the importance of the endothelium in mediating hypoxia-induced vasodilatation (135, 336, 472) *versus*, for example, that mediated by adenosine (24, 307). If hypoxia is involved, the consensus appears to favor prostaglandins as the likely mediators (56, 287), rather than NO (135) or endothelium-derived hyperpolarizing factor (EDHF) (136) (see later). Some evidence, however, suggests that the endothelium actually counteracts dilatation through release of vasoconstrictors in response to hypoxia (150, 418, 473, 477). Although these conflicting reports can partly be explained by differences in experimental protocol (*e.g.*, duration of hypoxic episode), this remains a controversial area.

B. Neurohormonal stimuli

A number of blood-borne messengers are potent stimulators of the endothelium, primarily *via* G protein–coupled re-

ceptors that ultimately evoke an increase in intracellular Ca^{2+}. Bradykinin is an important endogenous activator of endothelial cells (181), whereas catecholamines associated with sympathetic nerve activity also stimulate endothelial cells, primarily *via* α_2 and β_2 adrenoceptors (151, 240, 241). Acetylcholine (ACh) is the gold-standard stimulator of endothelial cells, particularly *in vitro*, where it is used extensively to study endothelium-dependent relaxation (133). However, although ACh is also used regularly in clinical studies, it is unclear to what extent ACh mediates endothelium-dependent vasodilatation *in vivo*, given that circulating anticholinesterases rapidly destroy any blood-borne ACh, and most blood vessels, with the exception of coronary (44) and cerebral arteries, lack parasympathetic stimulation. Nevertheless, muscarinic [mainly M_3 (33), but also other muscarinic subtypes (453,452)] ACh receptors are expressed on endothelial cells of at least some blood vessels, and their activation results in elevated endothelial intracellular calcium and activation of endothelium-derived relaxing factors. Other agents, such as serotonin (5-HT), histamine, and substance P, also act through their respective receptors to stimulate endothelial cells to release relaxing factors *via* calcium mobilization, but insulin, leptin, adiponectin, estrogen, and glucocorticoids, among others, act *via* their respective receptors to stimulate NO synthesis *via* a phosphorylation cascade, ultimately resulting in AKt-mediated phosphorylation of endothelial NO synthase (eNOS) (214) (Fig. 1).

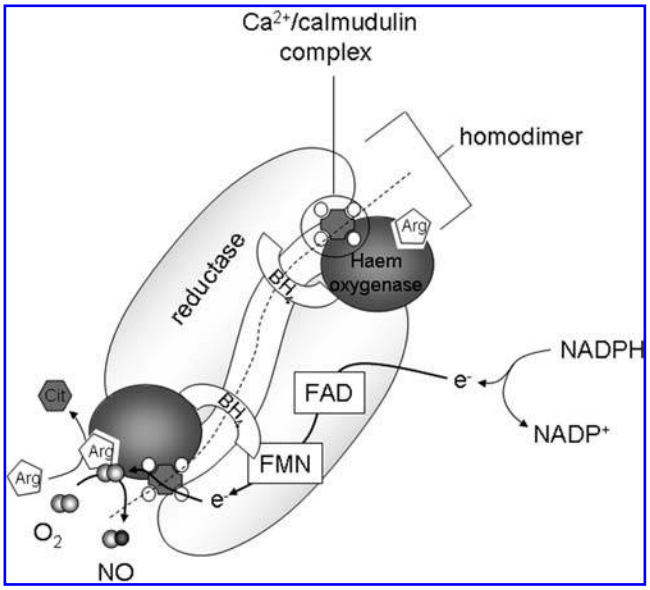

FIG. 2. Nitric oxide synthase (NOS) is a homodimeric enzyme, with heme oxygenase and reductase domains in both monomers. Catalysis of L-arginine to NO occurs in the oxygenase domain on binding of Ca^{2+}-calmodulin, which is thought to provide an electron bridge for transfer of electrons from NADPH *via* flavone nucleotides in the reductase domain; in iNOS, Ca^{2+}/CAM is permanently bound, hence the very high levels of activity. BH_4 is an essential cofactor for enzyme activity, probably helping to maintain the link between the two monomers, but also perhaps maintaining the heme iron in the high spin state necessary for activity and also offering some antioxidant protection (229).

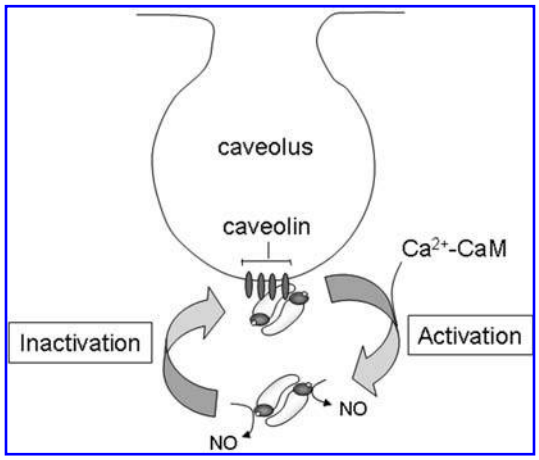

FIG. 3. Disassociation of NOS from caveolin is essential for enzyme activity.

III. Endothelium-Derived Relaxing Factors

Since the 1970s, the number of endothelium-derived relaxing factors (EDRFs) has proliferated, and it is now recognized that an increase in endothelial Ca^{2+} effects the release of several relaxing factors, the relative proportion of which varies enormously across the vascular tree. Principal among the known factors to date are NO, prostacyclin (PGI_2), and EDHF. NO is known to predominate in large conduit vessels like the aorta, whereas EDHF is dominant in resistance arteries (377); PGI_2 contributes less than the other two factors, and its impact is more consistent throughout the vasculature. The exception to this rule, however, is the coronary circulation, where EDHF appears to have a greater influence in the large coronary arteries than might be predicted, particularly in porcine models (54, 57, 134, 169, 338).

A. Nitric oxide (NO)

NO is a free radical species with powerful vasodilator properties as well as a number of other protective effects (295). It is synthesized from the amino acid, L-arginine, by NO synthases (NOSs) (225, 295). The endothelial isoform, eNOS (or NOS III), is constitutive and is predominantly, although not exclusively, found in endothelial cells. The enzyme is a homodimer, with each monomer containing a reductase and a heme oxygenase domain (134, 403) (Fig. 2). eNOS is a highly regulated protein at both transcription and functional levels. Full function of the enzyme is dependent on its existence as a dimer, disassociation with the membrane protein, caveolin (129) (Fig. 3), activation through calcium-calmodulin, and sufficient supply of substrate (L-arginine) and cofactors, most notably, tetrahydrobiopterin (BH_4) (4, 202). Activation of the enzyme results in the oxidation of L-arginine by molecular oxygen at the heme oxygenase, to generate NO and L-citrulline *via* the intermediate, N^{ω}-hydroxy-L-arginine (Fig. 4). NO is a small molecule that is soluble in both aqueous and lipid phases, allowing it to diffuse rapidly from its source and to cross membranes unimpeded. The primary target of endothelium-derived NO is the enzyme, soluble guanylate cyclase (sGC) (300), in cells within close proximity of the source, most notably, smooth muscle cells, platelets, and inflammatory cells. Activation of sGC by

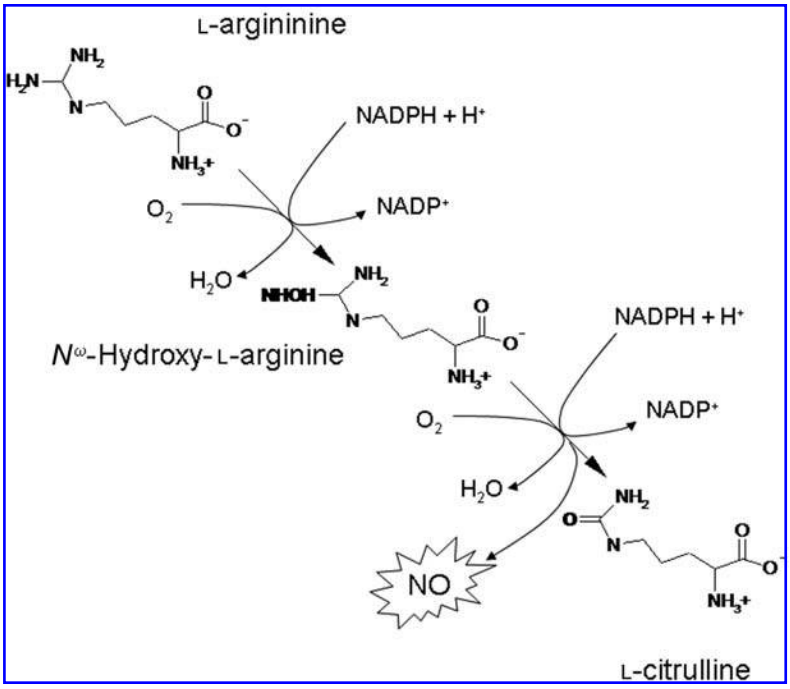

FIG. 4. NOS-mediated conversion of L-arginine to citrulline *via* N^{ω}-hydroxy- L-arginine, resulting in NO generation.

NO results in catalytic conversion of GTP to cGMP, which in turn mediates cell-specific effects *via* relevant cGMP-dependent protein kinases (PKs). In smooth muscle cells, PKG causes phosphorylation of myosin light-chain kinase, inhibits the inositol triphosphate (IP_3) pathway, and activates Ca^+ extrusion pumps, resulting in relaxation (300) (Fig. 5). Furthermore, in the chronic phase, cGMP inhibits smooth muscle mitogenesis *via* inhibition of the MAP kinase pathway by preventing *Ras*-dependent activation of Raf-1 (481). In platelets, the effect is to inhibit the activation processes involved in aggregation, primarily through impedance of Ca^{2+} mobilization and entry. Platelets themselves have constitutive eNOS (341), which is likely only to be stimulated upon platelet activation on account of the dependence of the enzyme on Ca^{2+} for activity; by definition, elevation of intraplatelet Ca^{2+} occurs only when the platelets are activated. The autocrine nature of platelet-derived NO, together with the fact that Ca^{2+} influx is both the stimulant for NO release and the target for its actions in this setting, can point only to NO as a regulatory brake on the activation process that serves to reduce the chance of inappropriate activation of platelets by low-level stimulants.

Two other isoforms of NOS are known: a constitutive neuronal isoform (nNOS, NOS I), which is a neurotransmitter in nonadrenergic, noncholinergic nerves in the peripheral nervous system and an inducible isoform (iNOS), which is usually expressed only in response to invading pathogens and inflammatory stimuli (225, 295). Regulation of iNOS is quite different from the constitutive isoforms; whereas eNOS and nNOS typically generate very low concentrations of NO (in the pico/nanomolar range) and are highly regulated by intracellular calcium, iNOS generates comparatively very high levels (μM range) of NO. The reason for the difference is that iNOS is typically expressed in inflammatory cells, and the NO generated is used in a noxious mix of chemicals designed to kill invading pathogens. Such high concentrations of NO, particularly when mixed with NAD(P)H oxidase-derived superoxide to generate highly toxic peroxynitrite ($ONOO^-$), hit many targets besides sGC in nearby cells, including respiratory chain enzymes (261, 353) and DNA (53, 409), with lethal effect. However, it is well recognized that iNOS induction is constrained neither to the infectious state nor to inflammatory cells, and its expression has been identified in a wide range of cell types in association with inflammatory conditions (361). It is also clear that iNOS does not necessarily produce "pure" NO; dysfunctional iNOS can generate $ONOO^-$ or superoxide or both and can therefore contribute to reduced endothelial cell viability, increased inflammation and peroxidation, and reduced endothelium-derived NO bioavailability.

The free radical nature of NO is critical to understanding its physiologic impact. Radicals are naturally regarded to be reactive species, and, although the high affinity of NO for the Fe^{2+} in the heme of sGC ensures that a proportion of NO is likely to find its target, it will inevitably react with other off-target molecules in cells. Principal among other reactants for NO are other heme groups (*e.g.*, in hemoglobin) (148), molecular oxygen (247), and oxygen-centred free radicals (82). The natural quenching of NO through inactivating reactions is an essential component in ensuring that its actions are localized to the vicinity of its production, but it is becoming evident that the ultimate fate of NO is also crucial in determining downstream activity of the moiety. For example, the simple oxidation of NO by oxygen to higher oxides of nitrogen generates powerful nitrosating agents in N_2O_3 and N_2O_4 that can go on to nitrosate cysteine residues in a wide range of proteins, altering their function (198); the reversibility of the nitrosation process means that *S*-nitrosothiols constitute a dynamic NO store, which can itself reflect endothelial function (282) and can be altered in, for example, hypertension (125). Equally, nitrite, once considered to be the inert product of NO oxida-

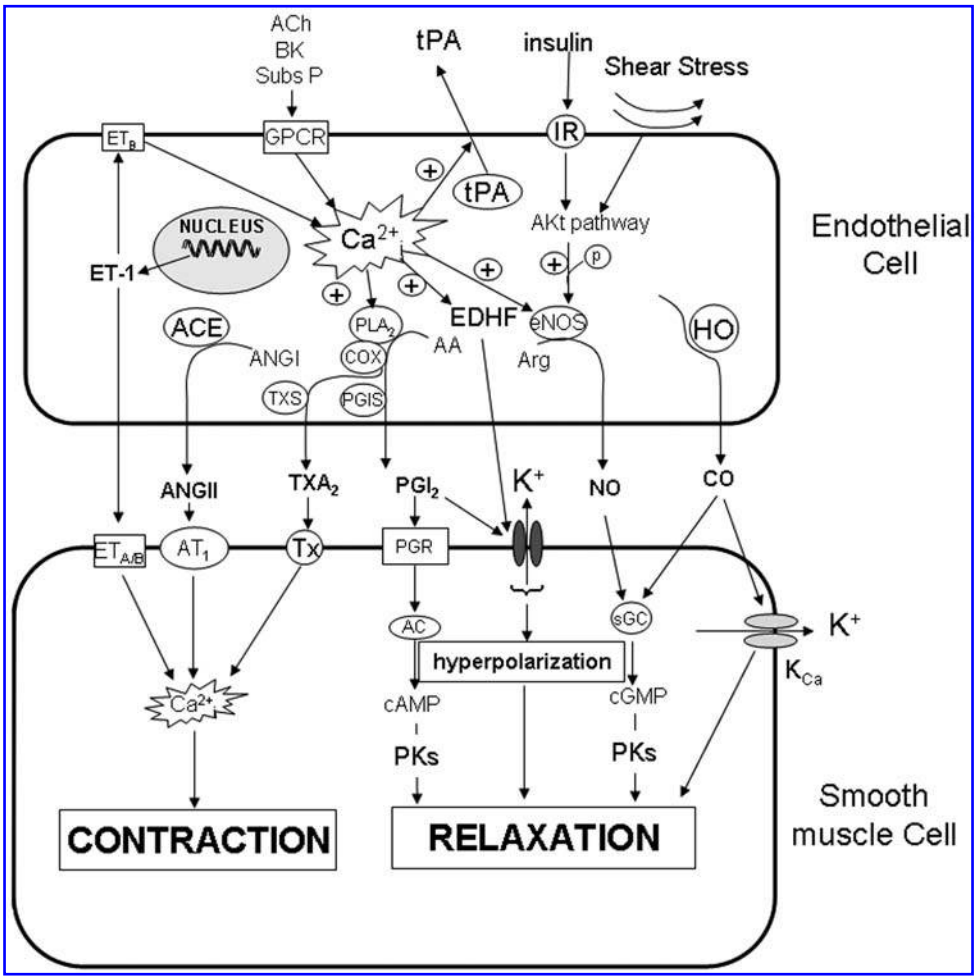

FIG. 5. Endothelial control of vascular smooth muscle function: an integrated system. Ach, acetylcholine; ANG II, angiotensin II; AC, adenylate cyclase; AT1, angiotensin receptor; BK, bradykinin; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monoophosphate; CO, carbon monoxide; ET-1, endothelin 1; ETNO, endothrelin A & B receptors; EDHF, endothelium-derived hyperpolariing factor; GPCR, protein coupled receptor; HO, heme oxygenase; NO, nitric oxide; PCI2, prostacyclin; PGIS, prostaglindin 12 synthase; PGR, prastaglandin receptor; PKs, protein kinases; sGC, soluble guanylate cyclase; tPA, tissue plasminogen activator; TXA2, thromboxane A2 VGCC, voltage-gated Ca2 channel.

tion, is now regarded as being a vasoactive substance in its own right (47, 141, 142).

Whereas the reaction of NO with oxygen at concentrations found in the vicinity of the endothelium is likely to be relatively slow, that with other radical species, and superoxide in particular, is considerably faster, irrespective of the generally low concentrations of the reactants. Generation of reactive oxygen species (ROS) is an inevitable consequence of several cellular processes, not least of which is respiration (321) (Fig. 6). In health, a battery of antioxidant defenses represents a formidable impediment to the prolonged existence of these potentially deleterious agents. Besides protecting cellular components from harmful peroxidation, rapid removal of ROS also protects NO from inactivation in both endothelial and target cells. However, in a wide range of disease states, oxidative stress can develop, whereby the existence of ROS is prolonged either because ROS generation has increased to levels that have swamped the antioxidant defenses, or because the defenses themselves have been downregulated, are dysfunctional or depleted. Clearly, oxidative stress is detrimental on a number of levels, but an immediate effect with respect to endothelial function is the inactivation of NO, most notably by superoxide (158). The interaction between these two radical species has led to NO being referred to as an "antioxidant" in some quarters on account of its ability to quench superoxide. However, in our opinion, this description is misleading with respect to NO, at least in the sense of direct inactivation of superoxide, because the product of the reaction, peroxynitrite ($ONOO^-$), is itself a powerful oxidant. Although not a radical, $ONOO^-$ is highly reactive, mediating lipid peroxidation (32) and nitration of tyrosine residues that can alter protein function (154). Far from being protective, as the term antioxidant implies, the reaction of NO with superoxide exacerbates endothelial dysfunction and contributes to the atherogenic process through the actions outlined earlier, together with cytotoxic activity in endothelial cells. Furthermore, $ONOO^-$ inhibits Ca^{2+}-activated K^+ channels in the smooth muscle of human coronary arterioles and contributing to impairment of EDHF-mediated relaxation of these vessels (260) as well as inhibiting PGI_2 synthase (497).

The combined effect of oxidative stress on endothelial function and atherogenesis is compelling; not only does it eliminate protective NO, but it also contributes to inhibition of EDHF-mediated vasodilatation, endothelial cell death, lipid peroxidation, as well some as yet relatively poorly evaluated effects on protein function through tyrosine nitration.

Although NO is commonly described as a "relaxing factor" for historic reasons, its greater importance in conduit rather than resistance arteries might suggest that its primary role lies in properties other than its powerful dilatory effects. Indeed, the platelet and inflammatory cell-directed effects

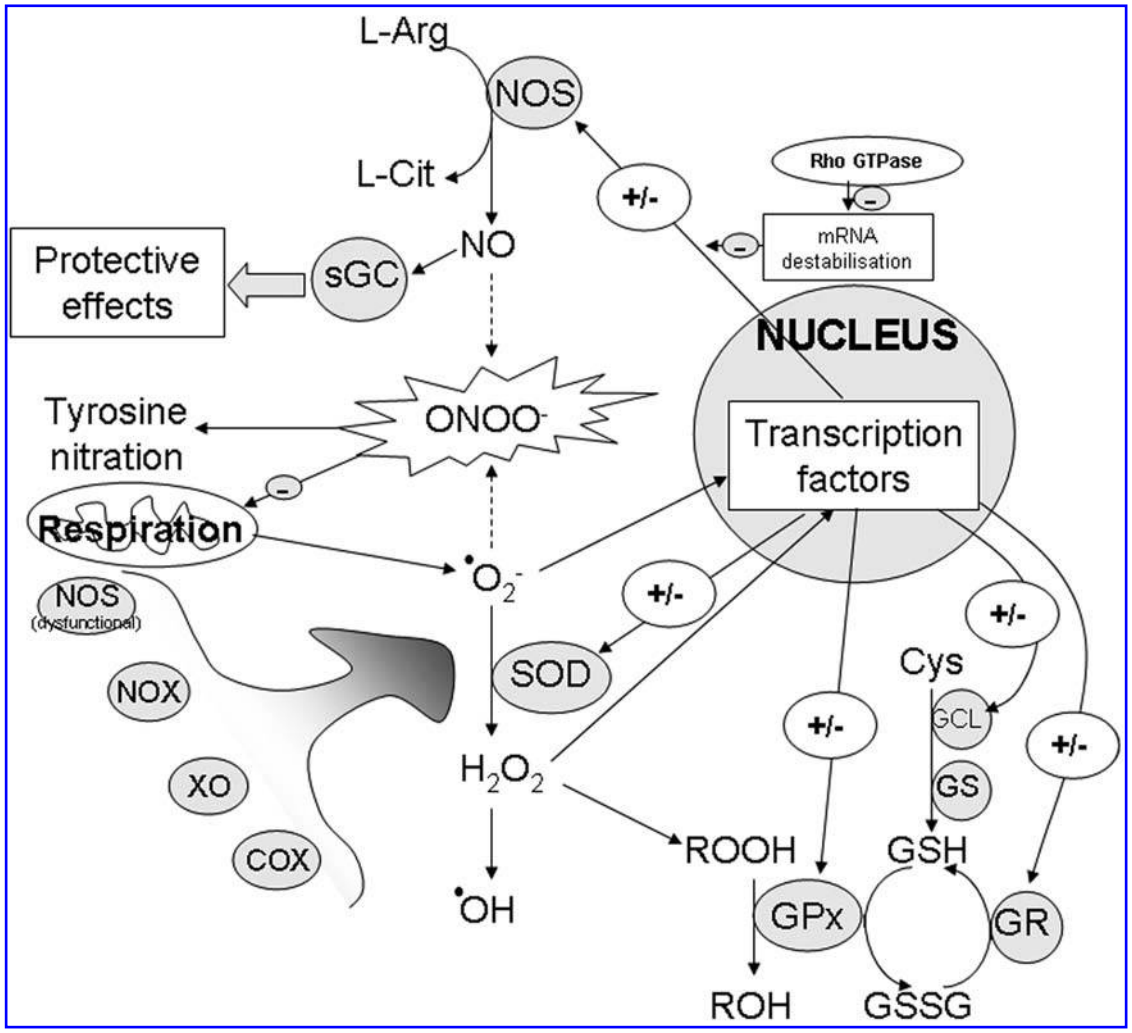

FIG. 6. Interplay between NO and reactive oxygen species (ROS). Levels of NOS and enzymes that control ROS levels are under tight control through transcription factor–mediated alterations in protein expression. COX, cyclooxygenase; GCL, glutamate-cysteine ligase; GPx, glutathione peroxidise; GR, glutathione reductase; GS-glutathione synthase; GSH , glutathione; GSSG, glutathione (oxidised form); MPO, myeloperoxidase; NOS, nitric oxide synthase; NOX, NAD(P)H oxidase,;sGC, soluble guanylate cyclise; SOD, superoxide dismutase; XO, xanthine oxidase.

noted earlier ensure that NO is a powerful antithrombotic and antiinflammatory agent, whereas its ability to also inhibit smooth muscle cell proliferation suggests a role in determining the structural composition of the vascular wall. Taken together, these properties provide NO with a unique antiatherogenic profile, which matches with its predominance in large conduits that are susceptible to atherosclerosis. The importance of the endothelium, and NO in particular, in protecting against atherosclerosis is further emphasized by the pivotal role played by denudation or dysfunction of the endothelium, or more specifically, dysfunction in the L-arginine/NO/sGC pathway, in the initiation and development of atherosclerotic plaques.

Dysfunction with respect to the NO/sGC pathway can occur at a number of levels (165). First, the endothelium might be absent (*e.g.*, after angioplasty) or the cells dead or dying in the face of toxic stimuli, including ROS. Second, enzyme expression might be altered *via* transcription factors or changes in stability of mRNA, mediated by rho-GTPases (351) (Fig. 6). Third, enzyme activity might be depressed on account of changes in the association with caveolin (114) (Fig. 3), reduced availability of substrate (L-arginine) (77, 81, 356) or cofactor (BH_4) (67), all of which are dependent on complex processes to determine intracellular levels (Fig. 7). Finally, the NO generated might be intercepted by, for example, superoxide under conditions of oxidative stress, compromising its biologic activity, while at the same time generating a prooxidant and highly cytotoxic by-product ($ONOO^-$) (Fig. 6). Therefore, even if endothelial dysfunction is traced specifically to this pathway, considerably more data are required to establish what is responsible for the dysfunction, before identifying a therapeutic approach. It is worth noting, however, that oxidative stress has multiple effects at different levels of this pathway, perhaps highlighting oxidative stress as a prime target with respect to therapeutic intervention in endothelial dysfunction.

B. Prostanoid vasodilators

The discovery of endothelium-derived prostaglandins pre-dated that of NO by several years and provided the first

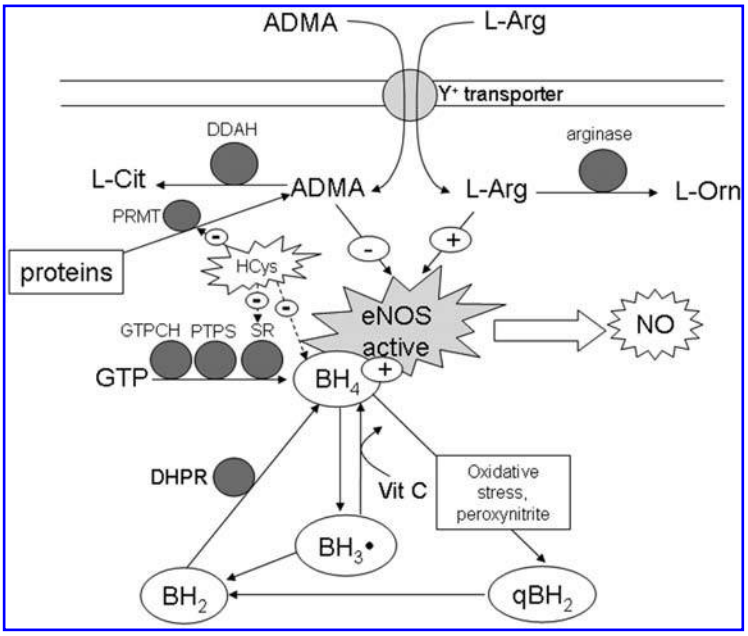

FIG. 7. Substrate and cofactor control of NOS activity: impact of homocysteine. ADMA, asymmetric dimethyl-L- arginine; BH4, tetrahydrobiopterin; DHPR, dihydrofolate reductase; eNOS, endothelial nitric oxide synthase; GTP, guanosine triphosphate; GTPCH, GTP cyclohydrolase; HCys, homocyst(e)ine; NO, nitric oxide; PTPS, 6-pyruvoyltetrahydrobiopterin synthase; SR, sepiapterin reductase.

indication of the endocrine role of the endothelium (441). Prostacyclin (PGI_2) is the primary endothelium-derived prostaglandin, although PGE_2 also can be generated. Like NO, the prostaglandins are synthesised on demand in response to an increase in intracellular Ca^{2+}, which activates phospholipase A_2 to generate arachidonic acid from phospholipids (Fig. 8). Endothelial cyclooxygenase(s) (COX-1 and possibly COX-2), together with endoperoxidases, convert arachidonic acid to prostaglandin H_2, which is finally acted upon by the relevant synthase to generate PGI_2 or PGE_2. The key role for Ca^{2+} in the process ensures that the stimuli for generation of these prostaglandins mirror those for NO, resulting in co-release of these agents. Although PGI_2 and NO also share the same vascular effects in terms of vasodilatation and inhibition of platelet and leukocyte function, the mechanism of action is quite different, with PGI_2 acting on cell-surface G protein–coupled receptors that activate adenylate cyclase, resulting in generation of cAMP (228). Dysfunction of the system can occur through endothelial denudation and alterations in expression or function of the synthetic enzymes involved. The relative importance of PGI_2 in endothelial function is eclipsed by the far greater literature relating to NO, but mounting evidence suggests that loss of the protective effects of PGI_2 through endothelial dysfunction also plays a critical role in vascular disease development. In particular, recent work relating to atherosclerosis suggests that PGI_2 is a powerful antiatherogenic agent (226), primarily through inhibition of leukocyte and platelet activation, whereas evidence from a knockout mouse model suggests that PGE_2 is proatherogenic, or at least that deletion of the gene coding for the synthase responsible for its synthesis leads to redirection of PGH_2 to protective PGI_2 (455).

C. Endothelium-derived hyperpolarizing factor (EDHF)

EDHF is a recently discovered endothelium-derived relaxing factor, which still has yet to be fully characterized [for review, see (73, 279)]. Many candidates have been proposed for EDHF, including epoxyeicosatrienoic acids (124), endogenous cannabinoids (317), C-type natriuretic peptide (362), or even hydrogen peroxide (230, 274, 275, 401, 480), among others, but the consensus is beginning to settle around a K^+-mediated event that could involve gap junctions (118, 123, 363), activation of which might involve any or all of the previously mentioned stimuli (Fig. 9). In our opinion, EDHF should not be considered to be a single "factor," but rather a combination of mediators and processes that are triggered on stimulation of endothelial cells, all capable of depressing intracellular K^+ in vascular smooth muscle. The balance of the mediators and processes involved is apparently very different, according to the specific blood vessel and species, which accounts for the conflicting evidence in the literature. Even this, however, is likely to be an oversimplification of the phenomenon, with evidence from human microvessels indicating that K^+ might not be involved (280).

The stimuli for EDHF-mediated vasodilatation are shared with those for NO and PGI_2, as is the absolute dependence on a healthy, functional endothelium. Given the relative propensity for EDHF in resistance, coronary arteries (39, 121, 178) and renal afferent arterioles (454), among others, its profile is increasing with respect to hypertension and coronary artery disease. Importantly, evidence suggests that EDHF may serve as a counterregulatory system that is upregulated in hypertension to compensate for reduced bioavailability of NO (386). This finding provides an interesting new insight into the interaction between the different EDRFs, giving the impression that their regulation is sufficiently sophisticated to allow them to substitute for each other in diseased states.

D. Carbon monoxide and hydrogen sulfide

Recently, another somewhat surprising candidate has emerged as a novel endothelium-derived relaxing factor. Carbon monoxide (CO) is a highly reactive molecule

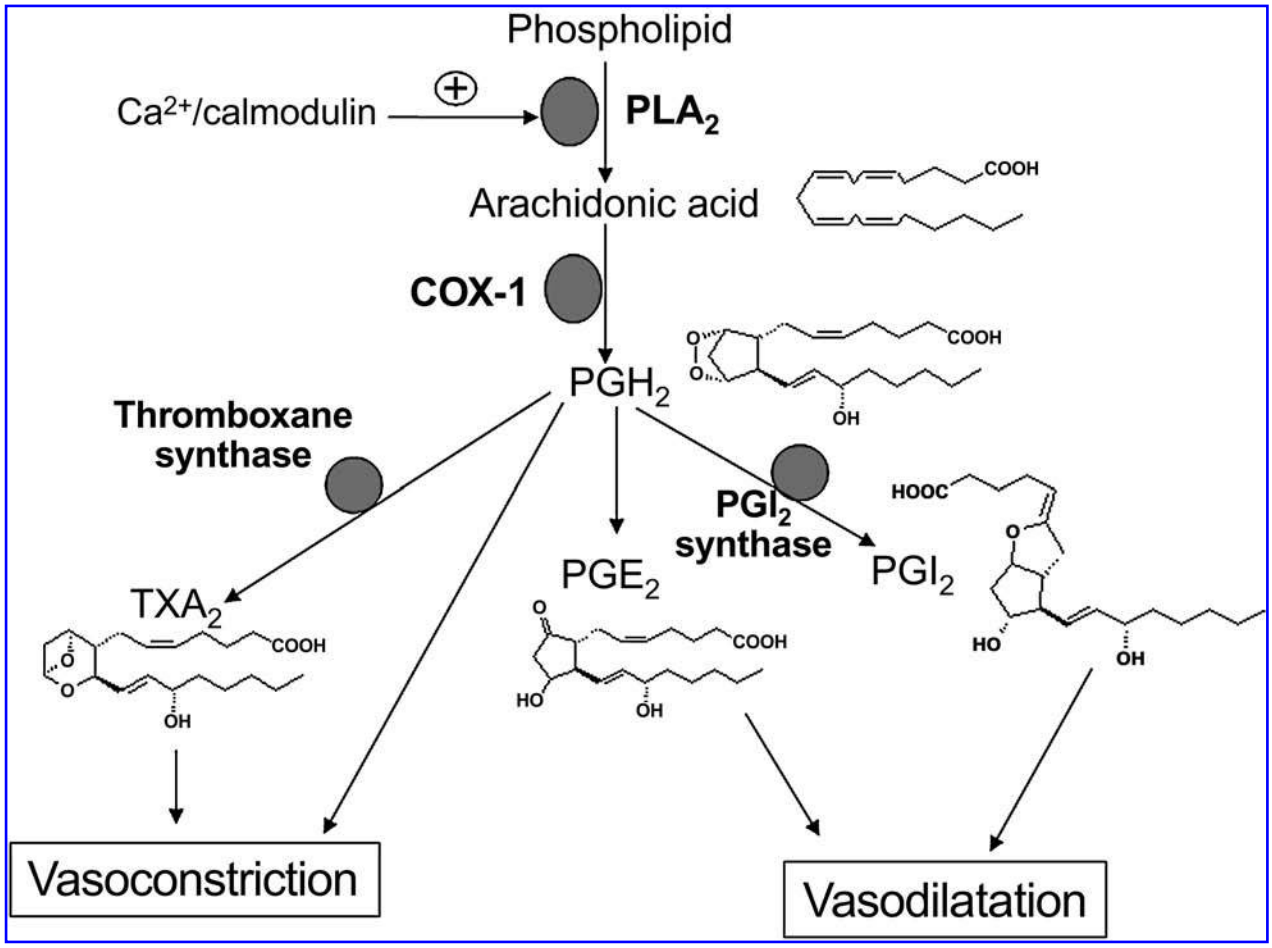

FIG. 8. COX-mediated synthesis of vasoactive prostaglandins. PLA2, phospholipase A2; COX-1, cyclo-oxygenase-1; PG, prostaglandin; TXA2, thromboxane A2.

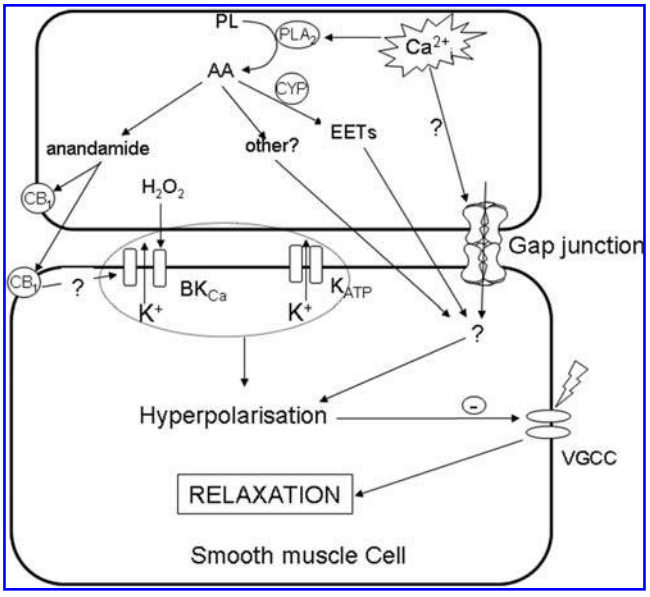

FIG. 9. Possible mechanisms of action of endothelium-derived hyperpolarizing factor (EDHF). AA, arachidonic acid; CB, cannabinoid receptor; CYP, cytochrome P450; EETs, epoxyeicosatrienoic acids; PL1%, phospholipase A2; VGCC, voltage gated calcium channel.

renowned for its poisonous properties on account of irreversible blockade of the heme groups in hemoglobin. It is now known that CO is generated endogenously during the conversion of free heme to biliverdin, by the action of a family of enzymes known as the heme oxygenases (HOs) (267). Both the constitutive (HO-1) and inducible (HO-2) isoforms have been found in the endothelium (305); HO-1 is upregulated in response to laminar flow *via* an ARE-mediated process (69). However, considerable evidence suggests that both isoforms can also be expressed in vascular smooth muscle cells. The roles of CO are largely analogous to those of NO, in that it is a relaxing factor (457) with antiproliferative effects on smooth muscle (188, 248), while also inhibiting adhesion of platelets (429) and inflammatory cells *via* activation of soluble guanylate cyclase (188) or a direct effect on K_{Ca} channels (457) (Fig. 5), or a combination of these. However, it is recognized that CO is a less-powerful stimulator of sGC than NO (132) and that it has a complex relationship with NO release and NOS expression (416). Nevertheless, CO has been shown to protect endothelial cells against apoptosis (19), most likely *via* cooperation with NF-κB (45), an important asset in protection against endothelial dysfunction. However, the precise impact of CO on endothelial function is disputed, with a number of studies also claiming that HO-derived CO is instrumental in promoting dysfunction in

animal models of several pathologic conditions, including metabolic syndrome and salt-induced hypertension (19, 196, 415).

The latest gaseous messenger to emerge in the cardiovascular system (and elsewhere) is hydrogen sulfide (H_2S). The balance of evidence to date suggests that the source of H_2S in blood vessels is smooth muscle rather than the endothelium (495), so it should not be regarded as an EDRF *per se*. Nevertheless, some evidence indicates that H_2S activates release of both NO and EDHF (183, 493), although an indication also exists that H_2S can downregulate NOS in the longer term (456). NO, in turn, appears to upregulate the enzyme responsible for H_2S synthesis in vascular smooth muscle, cystathionine γ-lyase (CSE) (495).

H_2S has been shown to induce vasorelaxation at physiologic concentrations (493), *via* activation of ATP-sensitive K^+ (K_{ATP}) channels (495), the opening of which hyperpolarizes cells and closes voltage-dependent calcium channels (238). Intravenous injection of H_2S decreases the mean arterial blood pressure of anesthetized rats by decreasing vascular resistance (495), and daily intraperitoneal injections of D-L-propargylglycerine (PPG, a specific blocker of CSE), for 2–3 weeks elevates systolic blood pressure, which may be a result of decreased endogenous H_2S production in vascular tissues (494). So it has been hypothesized that, by relaxing vascular smooth muscle cells, promoting apoptosis of smooth muscle cells (238) and inhibiting proliferation-associated vascular remodelling (238), H_2S modulates both the function and structure of the circulatory system (238).

The complexity of the involvement of H_2S is set to increase, given that its precursor, cysteine, is also central to synthesis of the intracellular antioxidant, GSH, together with its proposed interaction with homocysteine, a possible mediator for some forms of dysfunction.

IV. Endothelium-Derived Contracting Factors

Not long after the phrase "endothelium-derived relaxing factors" was coined, an equivalent term was established to describe humoral contracting factors (EDCFs), the identity of whch were not yet known. In time, as with EDRFs, a number of contracting factors have been identified, ranging from superoxide anion, which causes contraction, or more accurately, attenuates relaxation, by inactivating NO (see earlier), through to prostanoids and peptides (265).

A. Prostanoid EDCFs

As well as the prostanoids mentioned earlier that have vasodilatory properties, some are vasoconstrictors. The most prominent exponents of prostanoid-mediated vasoconstriction are PGH_2 and thromboxane A_2 (TXA_2; Fig. 8). The former is the primary product of COX activity and an intermediate in the formation of both PGE_2 and PGI_2, whereas TXA_2 requires the specific activity of thromboxane synthase enzyme. Both are found in endothelial cells, although it is likely that platelets in particular might represent a more prominent source. Both act on thromboxane receptors in smooth muscle to evoke contraction (Fig. 5).

B. Superoxide

An interesting twist to the role of prostanoid production in the endothelium-dependent modulation of vascular tone is the finding that activity of the COX enzyme itself can generate superoxide (76). The impact of superoxide generated in this fashion is twofold: first, it increases oxidative stress in the endothelial cell and thereby can inactivate NO; and second, it inhibits PGI_2 synthesis, without affecting that of contractile prostanoids. Of course, COX is not the only potential source of superoxide within endothelial cells, with the respiratory chain, NAD(P)H oxidases, NOS itself, and endothelin (see later) among a host of potential sources of superoxide, which, unchecked by endogenous antioxidant systems, can mediate vasoconstriction, primarily *via* inactivation of NO.

C. Endothelin

The endothelins (ET-1, -2, and -3) are a family of 21 amino acid peptides, of which ET-1 is the most abundant and the primary form found in the cardiovascular system (152). Similar to the endothelium-derived relaxing factors, ET-1 is not stored in endothelial cells; rather, it is synthesized *de novo* in response to a range of stimuli, including inflammatory mediators (*e.g.*, cytokines, TGF-β, hypoxia, low shear stress, thrombin, glucose, various hormones). Endogenous inhibitors include NO and PGI_2, and both the stimulator- and inhibitor-mediated pathways ultimately act on the promoter region of the ET-1 gene in the nucleus to modulate transcription of pre-pro ET-1 mRNA (Fig. 10). After translation into prepro ET-1, the peptide is cleaved to Big ET-1 and finally by endothelin-converting enzyme (ECE) to the mature peptide (Fig. 10). ET-1 activates G-coupled ET receptors, which elevate intracellular calcium *via* the phospholipase C pathway (439). Smooth muscle cells express both subtypes of the receptor (ET_A and ET_B), activation of which results in potent vasoconstriction with the downstream involvement of Rho-kinase (376). However, endothelial cells express only ET_B receptors, which cause an increase in endothelial calcium and activation of the endothelium-derived relaxing factors described earlier (Figs. 5 and 10). Therefore, although ET-1 release results in net vasoconstriction, the magnitude of the effect is blunted by its action on endothelial ET_B receptors, an effect that would be diminished by endothelial cell injury or dysfunction of the downstream relaxing factors. The effect of endothelial dysfunction is often exacerbated with respect to endothelin by the fact that some agents that reduce the activity of relaxing factors are concomitant stimulators of ET-1 synthesis (*e.g.*, superoxide). The interaction of ET-1 and oxidative stress is highly complex: not only does superoxide stimulates ET-1 synthesis, but ROS also mediates some of the downstream effects of ET-1 [*e.g.*, JAK-2 activation (16)], as well as being the ultimate product of ET-1–mediated proinflammatory effects [*e.g.*, increase oxidative stress through induction of COX-2 (406), activation of NAD(P)H oxidase (251), and mitochondrial dysfunction (426), which apparently have an important role in disease-mediated vascular dysfunction in disease (111)]. Furthermore, ET-1 is seen to be a proinflammatory agent that contributes to vascular remodelling in pathologic conditions (14).

ET-1 is expressed throughout the human vasculature, and expression is increased in atheromatous tissue (486). Furthermore, ET-1–activated NF-κB is a key player in the inflammation cascade (466). Thus, the endothelin system is implicated in the pathogenesis of atherosclerosis, and several clinical disease states, including systemic hypertension (168),

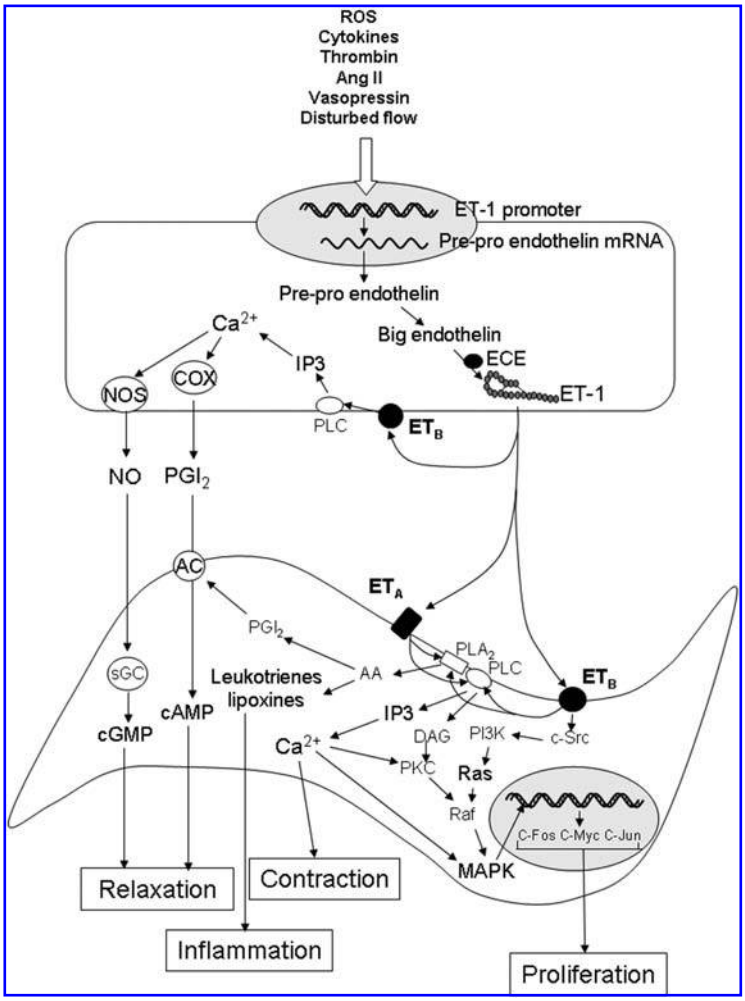

FIG. 10. Synthesis of endothelin (ET-1).

pulmonary hypertension (278), and chronic heart failure (217).

D. Angiotensin II (Ang II)

Ang II is another endothelium-derived vasoconstrictor peptide in the sense that the enzyme responsible for its synthesis from relatively inactive Ang I (angiotensin-converting enzyme; ACE) is found predominantly in the vascular endothelium (52). The primary action of Ang II is on AT_1 receptors, mediating a powerful vasoconstrictor effect in the acute phase (Fig. 5), but also leading to aldosterone secretion and sodium reabsorption in the kidney, the net effect of which is fluid retention and increased blood pressure. In the chronic phase, AT_1-receptor stimulation results in hypertrophy and hyperplasia in vascular smooth muscle and in cardiac myocytes, contributing to the remodelling of both blood vessels and the ventricles of the heart in chronic conditions such as hypertension and heart failure (42, 425). Further complexity in the role of Ang II is added by the fact that this peptide is well recognized to be a mediator of oxidative stress and endothelial dysfunction (94, 237), in part through upregulation of NAD(P)H oxidases [NOX (155,343); see Figs. 5 and 17 for the impact of NOX].

V. Tissue Plasminogen Activator (t-PA)

t-PA is an endothelium-derived fibrinolytic agent, the primary role of which is to dissolve rapidly thrombi that form in blood vessels. Synthesis of this serine protease is continuous but is stimulated by shear stress, thrombin, and histamine (163), and inhibited by plasmin (374) in a process that might involve protein kinase C (246). t-PA is stored free in the cytoplasm as well as in granules within endothelial cells; cytoplasmic t-PA is released constitutively, whereas that in storage granules requires elevated intracellular Ca^{2+} to mediate exocytosis. It is postulated that the primary stimuli for t-PA release are agonists related to the coagulation cascade, but other recognized stimulators of endothelial function (*e.g.*, bradykinin) are also known to stimulate release. Importantly, not all the recognized activators of the NO/sGC pathway and other endothelium-derived relaxing factors are capable of stimulating t-PA release; ACh and atrial natriuretic peptide are notable exceptions. The lack of consistency among agents that increase intracellular Ca^{2+} and stimulate endothelium-dependent vasodilatation to also cause t-PA release would suggest that the release mechanism is more complex than a simple Ca^{2+}-activated response, but it has yet to be fully elucidated.

The paradigm for t-PA function *in vivo* is that activation of the coagulation cascade associated with formation of microthrombi on an eroded atherosclerotic plaque, or a full-blown thrombus after plaque rupture, activates t-PA release as a counterregulatory measure designed to restore the affected blood vessel patency and minimize the detrimental effect of infarction. As with endothelium-derived relaxing factors, the effect of activated t-PA release is greatest in the locality of the stimulus, in this case, a thrombus; systemic dilution and inactivation by circulating plasminogen activator inhibitor (PAI-1) is sufficient to ensure that the impact on global hemostasis is minimized. The full extent of the anatomic distribution of tPA is not yet known, but early evidence suggests that it is most abundant in large conduit vessels (330, 360, 398), which is in keeping with the likely incidence of atherothrombotic disease. Moreover, many of these studies indicated that expression is enhanced in atherosclerotic vessels, indicating the ability of this system to anticipate the likely sites of thrombotic events. It is interesting to note that t-PA is most abundant in vessels dominated by NO, and some evidence suggests that NO might be involved in t-PA release.

The reduced ability of dysfunctional endothelium to generate t-PA is evident in a wide range of cardiovascular diseases, including hypertension (185) and coronary artery disease (313). Patients with hypercholesterolemia, in whom endothelium-dependent vasodilatation is affected, do not have impaired acute t-PA release (314). This finding is in keeping with clinical evidence that serum cholesterol levels do not influence the rate of patency of occluded vessels, but is further evidence that t-PA release involves complex mechanisms, not all of which are shared by other endothelium-dependent markers. The impact of smoking on t-PA is particularly interesting; basal plasma levels are often seen to be increased, but dynamic release of t-PA in response to a stimulus is dramatically impaired in smokers (313). The precise mechanism underlying the association is not yet fully explored, but oxidative stress is sure to underpin smoking-induced defects in t-PA responses.

VI. Endothelial Activation by Inflammatory Stimuli

Activation of the endothelium by inflammatory stimuli results in the expression of a wide range of proteins that alter its function significantly. Most notable among these are vascular cell-adhesion molecules (VCAM-1, ICAM), together with selectins that are specific for endothelium, platelets, and leukocytes (E-, P-, and L-selectin, respectively) (74, 85, 87, 96, 187). Typically, such activation would be associated with injury or infection and is central to the recruitment of platelets and lymphocytes to limit blood loss and to evoke a localized inflammatory response. A full inflammatory response ensues, involving neutrophils, lymphocytes, and macrophages under the guidance of T cells. As well as facilitating the recruitment of inflammatory cells, cytokines and other inflammatory mediators have a profound effect on the release of certain endothelium-derived mediators, including ET-1 (200), which has an immediate and powerful vasoconstrictor effect (190). In addition, cytokines induce expression of iNOS and consequently affect generation of NO and NO-related species (*e.g.*, $ONOO^-$). The process ordinarily ceases on resolution, and the adhesion molecules and selectin expression dissipate before complete resolution of inflammation. In the event that inflammation ensues for prolonged periods, secondary effects of both inflammatory cytokines and factors such as increased ET-1 and alterations in NO generation become apparent, particularly with respect to remodelling of the vessel wall (Fig. 11).

A. Atherogenesis

Endothelial activation of the type described is recognized as a form of dysfunction because it is central to the atherogenic process [for review, see (88, 207)]. In this setting, adhesion-molecule expression by endothelial cells is triggered by accumulation of oxidized lipoproteins (ox-LDL) in the subendothelial space (Fig. 11). Monocytes are captured and translocate through the endothelial cell layer, whereupon they differentiate into macrophages, and proliferate and ingest the offending ox-LDL *via* scavenger receptors. Unfortunately, this process is not easily resolved because the lipid-laden macrophages (now known as foam cells) accumulate, and their demise results in lipid deposition within the vessel wall–a fatty streak. The influence of inflammatory cells in the atherogenic process is continued through their impact on neointimal proliferation, matrix metalloproteinase expression, and fibrosis that mediate the progression of a fatty streak to a mature atherosclerotic plaque (256, 355). Ultimately, inflammation generally subsides to leave a stable plaque; chronically inflamed plaques are commonly regarded to be at high risk of rupture, leading to acute thrombotic events such as an acute coronary syndrome and stroke. The function or otherwise of the endothelial cells that overlie mature atherosclerotic plaques and their role in plaque rupture is largely unknown, but it is often surmised that it is dysfunctional. The unusual flow patterns and wall stresses caused by plaques might contribute to endothelial apoptosis (428) and erosion at critical points on the plaque surface.

Myeloperoxidase (MPO) is a leukocyte-derived heme-containing enzyme (315) that has been identified as having an important role in the atherogenic process (86, 335, 405). MPO is secreted on activation of leukocytes (neutrophils, monocytes, and some subtypes of tissue macrophages), whereupon it converts H_2O_2 into potent oxidants, including hypochlorous acid (HOCl) and nitrating species (218, 315). Clearly MPO is an important source of oxidants in inflammatory conditions, and its role in the etiology of atherosclerosis-related oxidative stress (Fig. 11) is well documented. It is apparent, however, that the effects of MPO on endothelial dysfunction are exacerbated by its transcytosization to the subendothelial space, where it is ideally placed to intercept NO through oxidative modification, resulting in enhanced nitrotyrosine formation in the immediate vicinity (315).

The numerous reactive oxidants and diffusible radical species generated from MPO (219) are capable of both initiating lipid peroxidation (490, 491) and promoting an array of posttranslational modifications to target proteins, including halogenation, nitration, and oxidative cross-linking (170, 335). MPO also has a role in oxidative modification of HDL, and it has been shown that apolipoprotein A-I (apoA-I), the primary protein constituent of HDL, is a selective target for MPO-catalyzed nitration and chlorination *in vivo*, resulting in inhibition of ABCA 1–dependent cholesterol efflux from macrophages (496).

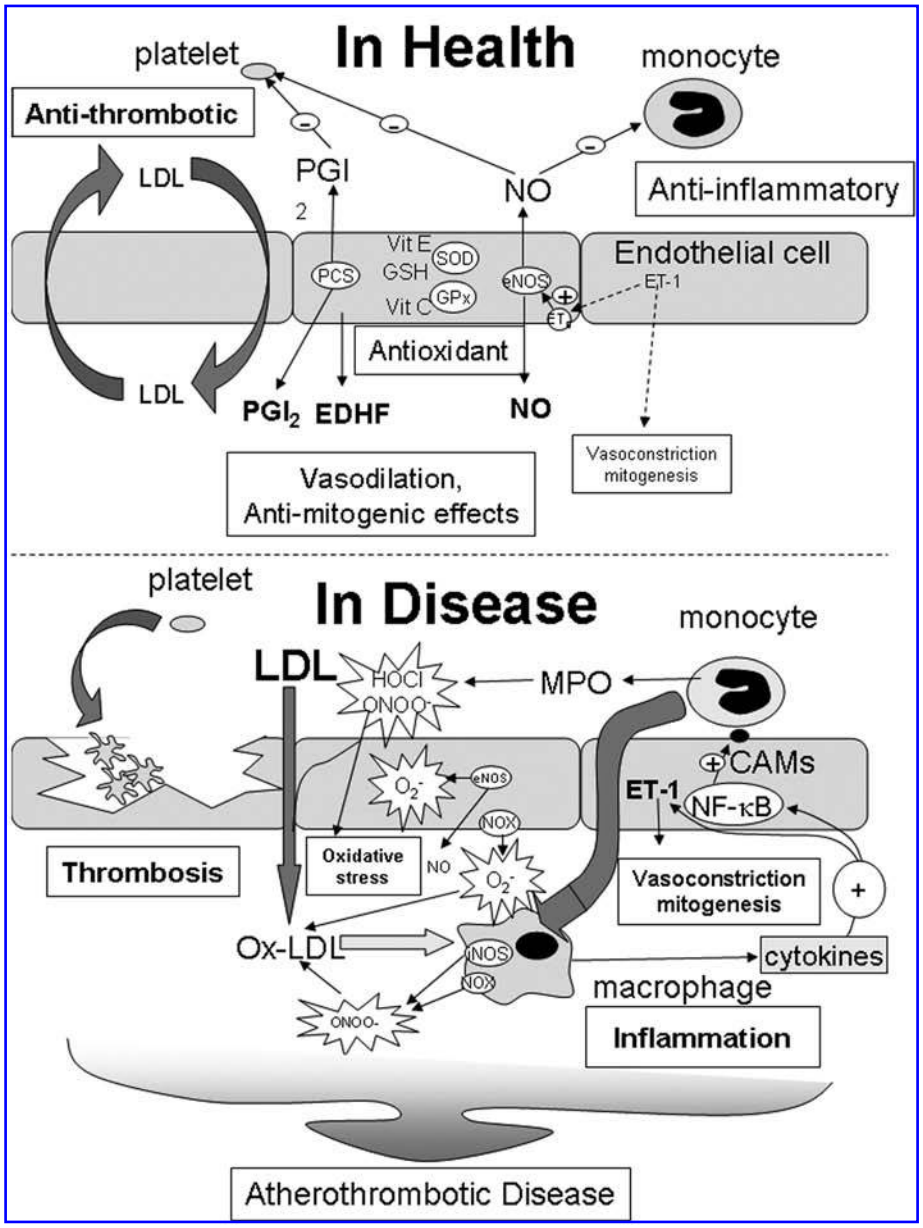

FIG. 11. The endothelium in health and disease: conversion from an anti-atherogenic, antioxidant protective "organ" to a pro-inflammatory, pro-oxidant entity that contributes to the atherogenic process.

Elevated levels of leukocyte and blood MPO have also been linked to the presence of coronary artery disease (CAD) in humans, highlighting a potential role for MPO as an inflammatory marker in CAD (489).

B. A pivotal role for NF-κB?

As with inflammation in other cell types, mobilization of nuclear factor-κB (NF-κB) is associated with the activation process in endothelial cells and with atherogenesis in general (75). Despite the lack of irrefutable evidence for a causal role for NF-κB in cardiovascular disease processes (largely on account of the lethal effects of gene knockout associated with the NF-κB pathway), the circumstantial evidence to support a pivotal role for NF-κB in the process is compelling. First, activated NF-κB is found in atherosclerotic plaques (38) but is all but absent from the surrounding tissue. Second, oxidative stress, inflammation, and dyslipidemia are central to the atherogenic process, all of which are reputed to activate the NF-κB pathway (36, 253). Finally, many of the risk factors associated with the atherogenic process, including hypertension (primarily through Ang II), diabetes (primarily as a result of hyperglycemia and advanced glycated end products; AGEs), hyperhomocysteinemia, and disturbed blood flow are linked to NF-κB activation. Activation of NF-κB stimulates expression of VCAM-1 and MCP-1 (75) (Fig. 12), both key players in the recruitment of inflammatory cells to the affected region. A sensory role for oxidative stress by NF-κB is disputed, particularly in endothelial cells, but the same article suggests a requirement for lipid peroxidation in the process instead (36).

C. Hyperlipidemia, oxidative stress, and inflammation: a noxious triad

Oxidative stress, hyperlipidemia, and inflammation are detrimental to cardiovascular health, even in isolation. How-

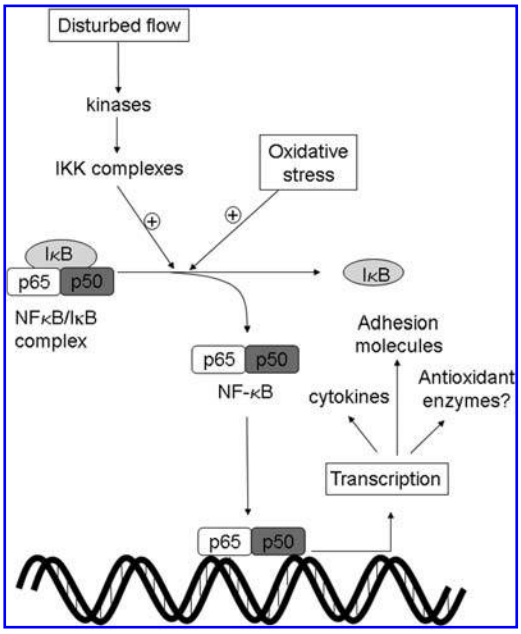

FIG. 12. The impact of NF-κB on inflammation and antioxidant control.

ever, the three are inextricably linked in the progression of endothelial dysfunction (58, 394, 399) and the resultant cardiovascular diseases. Oxidative modification of lipids plays a central role in propagating the inflammatory response and contributes heavily to the deposition of lipids in the vascular wall, whereas hyperlipidemia contributes to endothelial dysfunction and activation, which in turn leads to oxidative stress (256, 322) and inflammation. It is easy to see, therefore, that lipids, oxidative stress, and inflammation go hand-in-hand in the progression of atherogenesis and that all are legitimate therapeutic targets.

D. Environmental triggers of dysfunction: do infection and pollution play a part?

A broad range of risk factors (age, gender, smoking, diabetes, hypertension, hyperlipidemia, family history) have wholly explicable impacts on endothelial dysfunction and cardiovascular disease, but a proportion of clinical conditions related to endothelial dysfunction cannot be easily attributed to conventional risk factors. The discovery that plasma C-reactive protein is at least as good a predictor of cardiovascular events as plasma LDL (347) suggests that inflammation is correlated with disease progression. A number of studies have extrapolated this finding to a connection between bacterial infection and atherogenesis, with endothelial dysfunction perhaps providing the pivotal link (434). Some of the best data arise from work with *Helicobacter pylori* and *Chlamydia pneumoniae*: *H. pylori* has been associated with perivascular inflammation, specifically in coronary arteries, whereas *C. pneumoniae* has been found to be particularly prevalent in carotid endarterectomy specimens, in which it seems to predispose to thrombosis. Despite the fairly compelling retrospective data, however, meta-analysis of 15 prospective studies did not find an association between *C. pneumoniae* and cardiovascular disease, and the role for the endothelium in any association has not yet been fully examined (390).

A similar link has been proposed between pollution and atherogenesis, or plaque instability, or a combination of these. A number of clinical studies imply that exposure to diesel exhaust fumes, or the nanoparticles therein, is associated with increased risk of acute cardiovascular events, but the mechanism is still poorly understood (95, 290). Nevertheless, several preclinical studies have shown a link between pollution and endothelial dysfunction (162, 421) or inflammation (144, 475). In addition, recent clinical studies have indicated that endothelial function, as measured by responses to the endothelium-dependent vasodilator, bradykinin, as well as t-PA release, is impaired after acute exposure to relevant levels of diesel soot (291) and leads to increased risk of repeated myocardial infarction (451). Other clinical data point to an impact of pollution on different cardiovascular diseases, including hypertension and heart failure (43, 145). The implication is that pollution causes endothelial dysfunction, but the mechanism is unclear: is it caused indirectly *via* an increased inflammatory or oxidative stress response on deposition in the lungs, or do critical pollution particles enter the bloodstream as free entities or on-board macrophages and contribute directly to endothelial dysfunction? If the latter, is the impact of these particles purely toxicologic, or do they influence endothelial dysfunction through, for example, increased oxidative stress? The answers to these questions are critical in confirming the link between pollution and atherosclerosis or acute clinical events or both.

Inflammatory stimuli are emerging as independent risk factors for vascular disease, and the profile of the endothelium in mediating their effects in terms of disease progression is likely to increase as more research is conducted in this area. Furthermore, several studies that have investigated the effects of antibiotic therapy in patients at high risk of cardiovascular events have shown no reduction in cardiac events (110). Our opinion is that exposure to inflammatory stimuli might contribute to the atherogenic process, but what is more interesting is that these influences might well have an impact on plaque rupture, leading to myocardial infarction and stroke. The data relating to pollution are particularly interesting in this respect: epidemiologic data indicate that the incidence of myocardial infarction is increased within <1 day of exposure to pollutant particles (451), implying that pollution is associated with plaque rupture as well as endothelial dysfunction and atherogenesis. If true, it will be interesting to establish the cause of this association, especially in view of the rapidity of the effect.

E. Homocysteine and endothelial dysfunction

Homocysteinuria is the manifestation of an autosomal recessive disorder, in which patients have a defect of cystathione β-synthase, resulting in an increase in plasma homocysteine; these patients are at greater risk of premature coronary disease (107). Furthermore, in the general population, plasma concentrations of homocysteine appear to be associated with an increased risk of premature cardiovascular disease. Increased homocysteine levels are associated with endothelial dysfunction, vascular smooth muscle proliferation, increased thrombus formation, and inhibition of endogenous fibrinolysis (391), although short-term increases in

homocysteine through methionine loading do not, surprisingly, alter vascular stiffness (464), suggesting that the detrimental effects of homocysteine are slow to develop and are associated with prolonged elevation of homocysteine.

The cause of endothelial dysfunction seen in patients with homocysteinuria seems to include increased oxidative stress *via* impaired intracellular glutathione peroxidise-1 activity and inhibition of SOD, resulting in increased oxidation of LDL. Other mechanisms include increased apoptosis, increased ADMA (inhibiting NOS) (404), eNOS uncoupling through reduction of intracellular BH_4 activity (420) (Fig. 7), and decreased ICAM-1, VCAM-1, and E-selectin, which results in increased endothelial permeability and an increased risk of thrombosis (392). The prothrombotic effect of homocysteine might also be exacerbated by enhancement of activity and expression of factors XII and V and reducing the activity of protein C, thrombomodulin, and decreases the effectiveness of endogenous tPA. Platelet aggregation and activation are both increased by homocysteine, although the effect is not necessarily seen in brief exposure.

As noted earlier, most epidemiologic studies demonstrate an association between plasma homocysteine and increased cardiovascular disease (138). The magnitude of this effect varies between 20% and 80%. With the increasing mechanistic data suggesting a causal association, several randomized trials have been performed to test the hypothesis that pharmacologic reduction of plasma homocysteine will reduce CV risk. Homocysteine concentrations can be reduced by the simple intervention of vitamin B complex or folate. However, the current trials of these interventions have had mixed results; some studies have demonstrated improvements in endothelial function (97, 417, 467), coronary stenosis (371), and cardiovascular events (371), whereas others have shown no benefit (257, 445). One of the major issues when performing such trials is that the size of the effect of homocysteine is likely to be small when compared with more traditional risk factors, such as smoking and hyperlipidemia. Furthermore, clinically relevant reversal of endothelial dysfunction with agents such as folic acid is likely to be slow, and therefore, adequately powered trials must be large and have sufficiently long follow-up. Although the absolute cardiovascular benefit of homocysteine reduction is likely to be small, the population impact of a relatively safe intervention, such as vitamin B complex or folate supplementation, could yet be shown to be considerable in terms of reduced cardiovascular events.

VII. Experimental Measures of Endothelial Function

A. In vitro

1. Cell culture. Human umbilical vein endothelial cells (HUVECs) are the most readily available endothelial cells, although aortic, coronary, and resistance artery–derived endothelial cells from both human and animal sources are also commercially available. A vast literature exists relating to cultured endothelial cells and, in particular, the impact of oxidative stress and inflammation on the mechanisms underlying dysfunction. The advantage of using cultured endothelial cells is the potential for rapid throughput and in-depth investigation of cell signalling, but cell culture has its drawbacks. Endothelial cells undergo phenotypic changes, precluding their use beyond approximately passage 7 to 8, and even within this period, it is unclear how closely they truly resemble human arterial endothelial cells *in vivo*, not least because umbilical vein endothelial cells are (a) venous and (b) fetal in origin. Nevertheless, the data generated from endothelial cell culture has proved invaluable in dissecting out cellular mechanisms involved in endothelial function and dysfunction.

2. Functional assays. Traditional organ-bath pharmacology and myography continue to be a mainstay of *in vitro* analysis of endothelial function several decades after these techniques proved instrumental in the discovery of EDRF (133). The essence of these assays is a measure of endothelium-dependent relaxation of arterial segments in response to recognized agonists (*e.g.*, ACh, bradykinin). Instruments are available to enable force measurements in rings under passive tension (8), whereas others measure changes in vessel diameter in lengths of artery under flow conditions (perfusion myographs). The strength of these techniques is the total control of the environment and the ability experimentally to isolate elements of endothelial function (*e.g.*, through inhibitors like L-NAME and indomethacin, through removal of the endothelium altogether, or through induction of experimental oxidative stress). The technique can also be used as an *ex vivo* tool for determining the impact of disease development on endothelial function, experimental alterations in phenotype, and *in vivo* drug treatments in animal models and human vessels removed during routine surgery. It is important, however, to recognize the limitations of the artificial nature of *in vitro* functional analysis of this type in extrapolating results to the *in vivo* situation, not least with respect to the hyperoxic conditions under which most of these experiments are usually conducted.

An important issue relating to both cell-culture experiments and *in vitro* functional assays is the lack of physical stresses on the endothelial cells that would normally be experienced under flow conditions. Sophisticated experimental procedures are available to mimic at least some of these physical parameters [*e.g.*, shear-stress models for cell cultures (458), perfusion myography for blood vessel work (227), and isolated perfused organs (301)], but few, if any, can satisfactorily replicate the complex combination of shear stress and cyclical wall stretch that would be experienced *in vivo*. These should be important considerations in both experimental design and interpretation of results from studies using these models.

B. In vivo (clinical studies)

In vivo studies of endothelial function are fraught with difficulties, and currently no gold standard exists (the pros and cons of the most popular techniques are summarized in Table 1). Furthermore, many techniques that are purported to measure endothelial function actually measure "vascular reactivity," which is used as a surrogate for endothelial dysfunction. The ability of blood vessels to dilate to either external stimuli (*e.g.*, intraarterial infusion of, for example, ACh in forearm blood-flow experiments), or to the quasi-physiologic stimulus of reactive hyperemia (in flow-mediated dilatation), rely not only on the ability and function of the endothelium to release relaxing factors, but also on the innate ability of the blood vessels to dilate. The "stiffness" of blood

Table 1. Comparative Merits and Disadvantages of Available Methods for Assessing Vascular Function *In Vivo*

	Coronary artery studies	*Forearm venous plethysmography*	*Peripheral arterial tone (reactive hyperemia)*	*Pulse-wave analysis*	*Brachial artery reactivity (flow-mediated dilatation)*
Patient safety	+	++	+++	+++	+++
Validation of technique	++	++	+	++	++
Repeatability	++	+++	+	+	+
Ease of use	+	+	+++	++	++
Evidence of prognostic value	++	+++	+	++	+
Freedom from operator bias	++	+++	+++	++	++
Affordability	+	++	++	++	++
Use for repeated studies	+	+	+++	+++	++

+++, Technique performs well in this area; ++, intermediate performance; +, technique performs poorly.

vessels is, therefore, an important consideration alongside endothelial function; vessel stiffness is determined by a wide range of factors including age, smooth muscle cell hyperplasia, collagen cross-linking, glycation, and fibrosis. Thus, important structural and functional elements to vascular reactivity are often glossed over in the published literature and all but ignored in measures of "endothelial function." Furthermore, the potential dynamic and temporal interaction between endothelial function and structural vascular change are likely to differ significantly between different diseases, patient populations, and, indeed, individual patients. Although direct and comprehensive *in vivo* measurement of endothelial function is not, therefore, currently possible, an enormous literature relates to the impact of disease and treatments on "endothelial function" assessed by vascular reactivity. An appreciation of the different techniques and their limitations is therefore necessary to put study results in context.

1. *In vivo study techniques*

a. Measuring "endothelial dysfunction" in coronary arteries. In *vivo* coronary studies are expensive and difficult to perform in a large numbers of patients because of their invasive nature. However, the response of coronary arteries to ACh is particularly useful; it causes an endothelium-dependent dilatation in healthy coronary arteries (462) but a converse constriction in diseased vessels on account of a direct effect on ACh M_3 receptors on vascular smooth muscle (264, 423). Other endothelium-dependent vasodilators that have been used in the coronary circulation to measure endothelial dysfunction include bradykinin, substance P and 5-HT (146). Responses to intracoronary drugs can be measured by quantitative coronary angiography (197), intracoronary Doppler to measure flow (485), or by using intracoronary pressure wires (27). However, the benefits of direct measurements of coronary endothelial function must be weighed against their relative difficulty, expense, and the possible health risks to patients that are associated with these techniques.

b. Measuring "endothelial dysfunction" in peripheral arteries. Given that atherosclerotic plaque distribution is diffuse throughout the arterial tree, it is not surprising that endothelial responses in peripheral vessels correlate well with coronary artery responses (7). Assessment of peripheral arterial endothelial function has the advantage over direct coronary measures in that it is less invasive and, therefore, safer and less expensive. Peripheral vessel techniques are also more amenable to complex study protocols, giving more-detailed mechanistic data.

c. Venous occlusion plethysmography. Forearm venous plethysmography coupled with intraarterial drug administration can be used to investigate both endothelium-dependent (*e.g.*, ACh) and independent (*e.g.*, sodium nitroprusside, glyceryl trinitrate) vascular function and the direct vascular effects of vasoconstrictor and novel substances, which can be directly infused into one brachial artery, with the contralateral limb used as a control. Forearm blood flow can be assessed by strain-gauge plethysmography (244). However, this is still an invasive technique involving cannulation of the brachial artery, which is not without some risk (462). Although adverse clinical events are rare, this is a specialist technique that is not suitable for widespread clinical use.

Forearm venous plethysmography can also be coupled with reactive hyperemia (see later) to provide an indirect measure of endothelial function (176, 422). This may prove to be a useful clinical test in the future, but it less suitable for mechanistic studies.

d. Flow-mediated dilatation with brachial artery imaging. Brachial artery imaging coupled with reactive hyperemia is one of the most popular techniques for measurement of vascular function (62, 332, 387). Forearm or hand ischemia is induced by a tourniquet, release of which results in hyperemia of the distal vascular bed. This so-called reactive hyperemia is mediated by several factors that are released in response to ischemia, including NO (195), resulting in local vasodilatation and increased blood flow in both proximal and distal blood vessels. The increased blood flow in the proximal vessel (brachial artery) results in increased shear stress and an NO-mediated vasodilatation (flow-mediated dilatation; FMD). The magnitude of FMD is thought to be proportional to endothelial function, but structural alterations of blood vessels in disease and their ability to dilate are confounding factors. Another limitation is the degree of reactive hyperemia to the same stimulus, which is likely to vary in different disease states.

The brachial artery is the most commonly used vessel to study FMD, but other arteries, including the carotid (358),

have been used for this technique. However, although it is safe and relatively easy to perform, FMD is somewhat limited, in that it provides few mechanistic data. Furthermore, lack of standardization and variations in positioning of the arm cuffs and measurement of vessel diameter make comparing study results difficult.

e. Pulse-wave analysis and velocity. Large-vessel compliance decreases with advancing age and with classic cardiovascular risk factors, resulting in increased arterial stiffness and elevated systolic blood pressure. Although structural changes in the vessel wall are a major component of arterial stiffness (increased collagen and decreased elastin), the endothelium also appears to play an important dynamic role in arterial stiffness (216). Pulse-wave analysis and velocity represent two related techniques for assessing the propagation of the arterial wave form and reflected wave, resulting in a measure of arterial stiffness. High-fidelity tonometers are used to measure the peripheral arterial wave form, the data from which are further manipulated to establish an "augmentation index" [see (324) for review].

f. Doppler skin flowmetry. The technique of Doppler skin flowmetry relies on measuring the Doppler shift caused by moving red blood cells on reflected light of a known frequency. The signal (flux) is a product of the degree of Doppler shift (speed of red cells) and strength of signal (number of red blood cells) and thus a surrogate for blood flow. Changes in blood flow by reactive hyperemia (30), intradermal injection of substances (243), or iontophoresis of small charged molecules (80) have all been used to assess the skin microcirculation. The attraction of this technique is that it is relatively noninvasive, and even when coupled with intradermal injection, the technique is relatively safe, given the extremely small doses of study drug used (243). This technique is less technically challenging than measuring brachial artery diameter and, coupled with reactive hyperemic responses, may yet be developed into a clinically useful test of vascular "health." However, although vascular responses between the skin microcirculation and larger arteries are similar, whether skin blood-flow studies are representative of other vascular beds is unclear and requires further work.

g. Serum biomarkers for endothelial dysfunction. Use of serum biomarkers to determine endothelial dysfunction carries the advantages associated with routine sampling and straightforward measures. Many serum biomarkers have been shown to correlate with vascular disease and outcome, including interleukin-6, tumor-necrosis factor-α, soluble P selectin, and soluble intercellular adhesion molecule-1 (12, 349). The role of chronic inflammation in the atherosclerotic process continues to fuel the interest in hsCRP as both a mediator and a biomarker of atherosclerosis (348). CRP can influence a number of processes involved in endothelial dysfunction, including increasing ET-1 synthesis, downregulating eNOS (444), increasing the release of PAI-1 from endothelial cells (92), and influencing endothelial progenitor cells (444).

Cellular adhesion molecules (CAMs) are expressed on the surface of activated endothelial cells, and elevated plasma concentrations of soluble CAMs are seen in patients with atherosclerosis (331). Furthermore, vascular extracellular superoxide dismutase (SOD) is depressed in patients with coronary artery disease (234). Plasma nitrite is another measure that is increasingly popular in estimating endothelial function. Nitrite is a transient oxidation product of NO, which is rapidly converted to nitrate in the presence of red blood cells, but can be preserved in blood samples after rapid oxidation of red blood cell heme or immediate centrifugation and plasma freezing. The transient nature of the existence of nitrite ensures that its plasma level is a dynamic measure of endothelial NO generation, assuming that the rate of oxidation remains relatively constant. This measure is increasingly popular among clinicians, where it is used in vasodilator studies *in vivo* to confirm that any functional effect seen is reflected in modulation of NO synthesis (171, 221, 222). In addition, reduced ability of the endothelium to secrete t-PA on activation has recently been proposed as a reliable marker of endothelial dysfunction (325). The sensitivity of t-PA in this setting is apparently greater than that for other markers of endothelial dysfunction, including agonist- or flow-mediated vasodilatation, because it is measurable in some clinical conditions (*e.g.*, hypertension) in which agonist-induced vasodilatation is unaltered. Use of these biomarkers to measure vascular disease or prognosis is still at an early stage in development, but great potential exists in this field.

VIII. Endothelial Dysfunction and Aging

Aging is an independent risk factor for cardiovascular disease that affects us all. In keeping with enhanced risk of cardiovascular disease, clear evidence form both animal and human studies indicates that bioavailability of protective endothelium-derived factors (NO, prostacyclin, and EDHF) declines with age in both conduit and resistance vessels, with a concomitant increase in generation of, and sensitivity to, constrictors. Although the link between aging and dysfunction of the endothelium is undisputed, the mechanism(s) involved in the phenomenon are less clear; for example, eNOS expression has been shown to decline with age in some studies (18, 65, 84, 413, 430), but to increase in others (143, 437). It seems likely, however, that oxidative stress is once again the key to the association between aging and endothelial dysfunction on account of depression of antioxidant enzymes (*e.g.*, MnSOD) in response to prolonged exposure to ROS (437). The endothelium itself appears to be an important source of ROS that mediate downstream effects (143), with increasing dysfunction of mitochondria and other well-recognized sources of ROS (*e.g.*, xanthine oxidase, NOX) (71, 137) implicated in age-related dysfunction. ROS and a loss of NO are also likely regulators of conversion of endothelial cells to the senescent state, together with a reduced capacity to regenerate and replace endothelial cells that have undergone apoptosis, necrosis, or removal by erosion. This effect is, in part, due to a reduction in number of circulating endothelial progenitor cells, as well as a reduced ability of such progenitor cells to engraft and to develop full endothelial function, particularly with respect to eNOS. *In vivo*, an important stimulus for the expression of eNOS is the shear stress generated by flowing blood on the endothelial surface (344), which increases eNOS mRNA and stability (89) and may explain why physical training improves eNOS expression in older humans (410) and animals (413). Several other factors, such as estrogens (223) and growth factors (34), also can upregulate eNOS expression.

Secretion of many growth factors and hormones declines with age. Of these, estrogens and dehydroepiandrosterone (DHEA) have received the most attention, and DHEA has become a widely used antiaging drug. Hard scientific evidence has not been presented justifying therapy, although treatment of middle-aged men with hypercholesterolemia has been shown to improve endothelium-dependent flow-mediated dilator responses (206), but it is unknown whether long-term supplementation is able to prevent aging-induced endothelial dysfunction. Evidence exists in rats that long-term inhibition of the renin–angiotensin system can ameliorate endothelial dysfunction associated with aging through inhibition of COX-2–derived vasoconstricting factors and superoxide anion synthesis (299).

Taken together, it is evident that age-related changes in endothelial phenotype and function are detrimental to cardiovascular health and, indeed, mirror many of the processes that are associated with other risk factors predisposing to atherosclerotic disease. The inexorable march toward a dysfunctional endothelium is almost certainly a feature of even the healthiest individuals, but the rate of decline is heavily influenced by lifestyle; poor diet, smoking, and weight gain top the list for aspects of lifestyle over which individuals might exert some control in an effort to slow disease progression. Whether we will ever succeed in slowing the underlying ("basal") aging process is debatable, but a thorough understanding of the mechanisms involved in the process will no doubt shed further light on cardiovascular disease processes that might simply be viewed as accelerators of, or extensions to, the natural aging process.

IX. Endothelial Dysfunction in Cardiovascular Disease

Considerable interaction occurs between different disease states such as diabetes, vascular disease (coronary, cerebral, renal), systemic hypertension, pulmonary hypertension, and chronic renal impairment, and many bold conclusions from clinical studies about specific patient groups studied do not take this into consideration.

A. Atherosclerosis, coronary artery disease, and stroke

Atherosclerosis is a chronic, systemic disease. Thus, the majority of patients with clinical peripheral vascular disease will also have coronary disease and *vice versa*; both groups are also at increased risk of stroke.

The degree of coronary endothelial dysfunction appears to be prognostically important. Impaired endothelium-dependent coronary artery vasodilatation is associated with increased risk of subsequent vascular events (161, 414). The same is also true for impaired flow-mediated dilatation (366) and cold-pressor test (367). These findings appear to be consistent for patients with severe obstructive coronary disease, as well as for those with angiographically normal coronary arteries (*i.e.*, impaired function without encroachment of atheroma on the vessel lumen is associated with a poorer clinical outcome in terms of future events, compared with patients with normal coronary artery responses). Indeed, most studies in patients with clinically significant coronary disease or stroke measure endothelial dysfunctions in the peripheral vasculature, as these correlate well with coronary artery responses (7).

Plaque instability is a key aspect in the development of an acute coronary syndrome in which an acute inflammatory process increases the likelihood of plaque rupture (256). Endothelial dysfunction is associated with increased oxidative stress and inflammation (308). Plaque inflammation and rupture is a complex process that is incompletely understood but regulated in part by NO (304) and NF-κB (17). Atherosclerotic coronary arteries are prone to vasoconstriction, resulting in Prinzmetal angina. Furthermore, coronary artery spasm is commonly found at the site of plaque rupture and is a major contributor to the reduced blood flow and occlusion of coronary arteries during myocardial infarction. It is likely that this is a result of local release of vasoconstrictors, such as ET-1, but also to the impaired ability of atherosclerotic arteries to vasodilate (216, 486).

Treatments that may improve endothelial function systemically, such as ACE inhibitors and statins, appear to provide protection from acute clinical cardiovascular events, with significant improvements in morbidity and mortality. Although endothelial function is not yet easily measured clinically, it may in the future act as a guide to the effects of therapies on generalized endothelial function and allow tailored therapy for patients with clinically significant atherosclerosis.

The role of endothelial dysfunction in stroke does not appear to have attracted the same level of interest as that in coronary artery disease, although the reason for the relative paucity of data is not clear. Certainly one would anticipate that atherosclerosis-related stroke at least would be associated with endothelial dysfunction, and some evidence supports this notion [see (117) for review].

B. Diabetes

Oxidative stress is central both to the progression of type II diabetes [comprehensively reviewed by (63,113)] and to the cardiovascular consequences of both type I and type II diabetes. A wide range of sources of oxidative stress are found in diabetes and in prediabetic states [insulin resistance (184), metabolic syndrome (334)], including inflammation, dysfunctional cellular respiration, downregulated antioxidant defenses, and the impact of advanced glycated end-products (AGEs).

The mitochondrial respiratory chain is a major site of production of ROS within cells, with superoxide being produced continually as a by-product of normal respiration during synthesis of ATP (66, 342). Superoxide from mitochondria can initiate a range of damaging reactions, from the direct actions of the anion itself to the production of hydrogen peroxide (443), hydroxyl radical, and peroxynitrite, which can damage lipids, proteins, and nucleic acids (193). It has been suggested that production of mitochondrial ROS and subsequent oxidative damage during hyperglycemia may be central to much of the pathology of diabetes (46, 319), not least because the function of the mitochondrion itself is particularly susceptible to oxidative damage, and in the pancreatic β-cell, it plays a central role in glucose-stimulated insulin secretion (359). This leads to a vicious cycle in the progression of the disease, in which hyperglycemia leads to oxidative damage, disrupting the β-cell response to increases in blood glucose, and leading to further hyperglycemia (153).

As would be expected, mitochondria have an extensive range of antioxidant defenses, from Mn-SOD, which converts superoxide to hydrogen peroxide, to its own isoforms

of glutathione peroxidase and thioredoxin-dependent enzyme peroxiredoxin III, both of which detoxify hydrogen peroxide (79). The mitochondrial glutathione pool is distinct from that in the cytoplasm and is maintained in a reduced state by an isoform of glutathione reductase, which requires NADPH (79). Within the phospholipid bilayer, lipid-soluble antioxidants vitamin E and coenzyme Q both help to prevent lipid peroxidation (266), and an isoform of phopholipid hydroperoxide glutathione peroxidase degrades lipid peroxides within the mitochondrial inner membrane (79). A range of mechanisms repair or degrade oxidatively damaged lipids, proteins, and DNA (21). Nevertheless, oxidative damage is inevitable because some ROS produced by the mitochondria evade detoxification, leading to a steady-state level of damage, which is dependent on the relative rates of damage accumulation, repair, and degradation (193, 380).

The proposed consequences of hyperglycemia of particular pathologic relevance to mitochondrial dysfunction in diabetes are formation, autooxidation, and interaction with cell receptors of AGEs; activation of various isoforms of protein kinase C (PKC); induction of the polyol pathway; and increased hexosamine flux [reviewed in (153)]. Many of these pathways have been associated with oxidative stress, and one hypothesis is that all of these processes are a consequence of overproduction of superoxide by the mitochondria during hyperglycemia (46, 98, 319); however, the validity of this link has not yet been demonstrated. The evidence in support of this argument comes from experiments in cultured endothelial cells, in which increased glucose concentration increases cytosolic ROS production, activation of NF-κB, formation of AGEs, and activation of PKC, all of which were blocked with Mn-SOD, respiratory inhibitors, or uncoupling protein-1 (319). However, this link may turn out to be some other interaction with the mitochondrion, not mediated directly by the redox state of electron carriers (153).

Although it is tempting to suggest that the increase in mitochondrial ROS in response to hyperglycemia is the proximal defect that leads to most other pathologic consequences of the condition, this is probably too simplistic and will have to be extended to accommodate other sites of ROS production (461). However, it does suggest that prevention of overproduction of superoxide by mitochondria, or an increase in the rate of decomposition of such toxic molecules by antioxidants, may alleviate many of the pathologic consequences of hyperglycemia (153). Only a very small proportion of natural and artificial antioxidants will reach the mitochondria, and so antioxidants that are targeted to accumulate within the mitochondria may offer more protection (153). Derivatives of the natural antioxidants, vitamin E and coenzyme Q, specifically designed for this purpose, have shown some promise in *in vitro* studies, rapidly and selectively accumulating in isolated mitochondria and in intact, isolated cells (104, 210, 384). Another possibility for treatment is uncoupling proteins, such as 2,4-dinitrophenol (DNP), which has been used extensively in the past to treat obesity in humans (164), but unregulated administration, abuse, and a very narrow window between efficacy and toxicity led to abandonment (164). Also, one of the problems associated with uncoupling proteins is the decrease in the membrane potential of the mitochondrion, which in β-cells would make insulin secretion less responsive to plasma glucose levels, which would be counterproductive (153). Confirmation of their effect has been shown in animal studies, where treatment with DNP causes hyperglycemia (381).

AGE formation has been linked with several of the long-term complications of diabetes, including micro- and macrovascular disease (180, 277, 448). plasma levels of N^{ε}-(carboxymethyl)lysine (CML) and pentosidine double in patients with advanced diabetes (412). The mechanisms by which AGEs affect vascular function include formation of AGE-modified LDL (130, 450). In addition, a specific transmembrane AGE receptor (RAGE) initiates a cascade of events, including activation of NAD(P)H oxidase and a range of proinflammatory mediators (cytokines and vascular cell adhesion molecule 1; VCAM-1) (459). The consensus is that AGE–RAGE interaction is central to the cellular and vascular dysfunction associated with diabetes complications (231, 476), but some dispute exists in the area, because AGE treatment of HMEC-4 cells did not induce an inflammatory mRNA profile (435). The precise mechanism of signal transduction from RAGE to NF-κB–induced cytokine secretion remains largely unknown, although several reports have implicated p21 Ras, extracellular signal-regulated kinases (ERK) (479) 1 and 2 (231), and protein tyrosine kinase (PTK) in the effects (476). p38 Mitogen-activated protein kinase [MAPK; responds to cytokines and cellular stress inducers (479)] has also been shown to be a key downstream effector of RAGE in THP-1 monocytes (479) and is required for NF-κB transcriptional activation and subsequent increased secretion of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β), and macrophage chemoattractant protein-1 (MCP-1) (479). The ligation of RAGE has been shown to induce acute tyrosine phosphorylation, followed by either dephosphorylation or degradation, but no consensus kinase motif has been identified in the RAGE intracellular domain, and so it has been suggested that it probably couples with tyrosine kinases directly or indirectly to mediate the observed tyrosine phosphorylation (479).

A number of other receptors for AGEs have been identified, such as lactoferrin (370), oligosaccharide transferase complex protein-48 (also know as AGE-R1) (478), 80K-H protein (AGE-R2) (478), galectin-3 (AGE-R3) (449), lysozyme (254), macrophage-scavenger receptors (10), and CD36 (323). Increasing evidence also shows that Amadori-modified proteins have biologic effects very similar to those of AGEs, and they may also have their own receptors, which are different from all AGE-binding proteins, such as calnexin (shown on mesangial cells) (468), and nucleolin (nucleophosmin and cellular myosin heavy chain) (41), as specific binding proteins for fructoselysine on various monocyte-like cells. Binding of fructoselysine to these cells induces phosphorylation and activation of p38 and p44/42 MAPK, together with NF-κB activation (40).

An important aspect of tissue damage and cell death associated with chronic hyperglycemia and diabetes is mediated by ROS (459). Oxidative stress in this setting leads to oxidation of sugars, nonsaturated fatty acids, and glycated proteins, which causes an increase in glucose autoxidation and a depression of endogenous antioxidants (25, 459). Pentosidine and CML are AGEs of particular interest in the study of oxidative stress in diabetes, as both are produced by glycation and oxidation (298). It has also been shown that su-

peroxide anions and hydrogen peroxide are directly formed through the Maillard reaction (328), although the AGE/RAGE interaction facilitates ROS production, potentially leading to apoptosis of cells and compromised cardiovascular function (46).

AGEs have also been shown to increase the susceptibility of low-density lipoprotein (LDL) to oxidation (48), and this oxidized LDL is responsible for decreased NO production, by downregulation of NO synthase (459), contributing to defective vasodilation in animal models of diabetes (49).

From the inflammatory perspective, it has been shown that treatment of human inflammatory cells with high glucose (159, 372) or specific AGEs (383, 450), leads to oxidative stress and generation of proinflammatory cytokines. AGEs have been shown to augment the inflammatory response and to upregulate cyclooxygenase-2 (COX-2) *via* RAGE, which leads to monocyte activation and vascular cell dysfunction (372). AGEs have also been shown to lead to NF-κB activation in a process that may or may not be entirely RAGE dependent (302). Cells exposed to AGEs have been previously shown to have altered proinflammatory phenotypes; genes affected include those that encode for IL-1β (447), TNF-α (292), IL-6 (368), platelet-derived growth factor (PDGF) (218), insulin-like growth factor (IGF)-1 (218), thrombomodulin (369), vascular cell adhesion molecule (VCAM)-1 (369), and tissue factor (TF) (25). The interaction of hyperglycemia, AGEs, oxidative stress, and inflammation are summarized in Fig. 13.

Oxidative stress and endothelial dysfunction go hand-in-hand in mediating the onset and progression of diabetes-induced atherosclerosis, which is ultimately the greatest single factor responsible for premature death in patients with diabetes. Clinically, endothelial dysfunction is associated with insulin resistance (179, 214, 281, 378, 395) (Fig. 1), the manifestations of which are in part mediated by increased endogenous ET-1, leading to increased basal vasoconstriction (60, 273), as well as reduced activity of eNOS (126) and iNOS (270, 272). Increased plasma AGEs are apparently associated with endothelial dysfunction in humans (412), an effect that might be mediated *via* quenching of endothelium-derived NO (49). However, it is as yet unclear whether the association found is causal.

The underlying pathophysiology that underpins the reciprocal relationship between endothelial dysfunction and insulin resistance is comprehensively reviewed elsewhere (214). Clearly, the interaction between the processes is highly complex, involving inflammation, oxidative stress, and glucose toxicity. It is worth noting, however, that the AKt signaling cascade is central to the expression and activation of eNOS (Fig. 1), as well as the translocation of glucose transporters (GLUT-4) that help to maintain healthy function with respect to the endothelium and to insulin sensitivity. Evidence is amassing to suggest that defects in this pathway, driven by IKKβ in response to inflammatory stimuli, could mediate both pathophysiologic processes and might represent the key to the reciprocal nature of endothelial dysfunction and insulin resistance. Modulators of this pathway could provide an effective means of reversing the relentless progression toward diabetes and cardiovascular disease once insulin resistance takes hold. Synthetic peroxisome proliferator–activated receptor-γ (PPAR-γ) ligands (known as glitazones) are effective insulin sensitizers that appear to improve endothelial function (209). The impact of glitazones on the endothelium is multifactorial, with evidence to support enhancement of the PI3-kinase pathway, increased expression of adiponectin and antiinflammatory effects, as well as depression of MAP-kinase–mediated ET-1 secretion [see (214) for review]. It is somewhat baffling, therefore, that one such PPAR-γ ligand, rosiglitazone, has recently been at the center of a concern surrounding increased cardiovascular risk (320), although the findings of this meta-analysis have received heavy criticism in the literature.

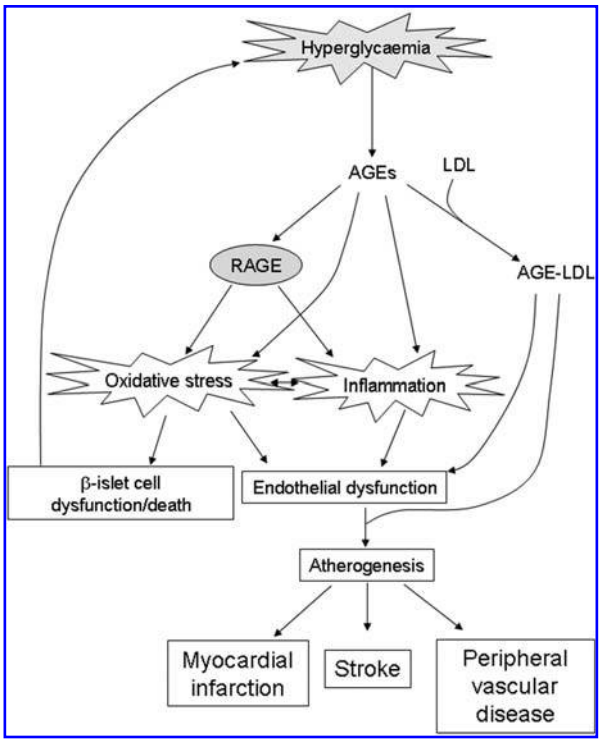

FIG. 13. Role of AGEs in translation of hyperglycemia into endothelial dysfunction and macrovascular disease.

This area of research is still at a fairly early stage in development, with many of the pathways still to be fully elucidated, but clear indications suggest that specific AGEs represent a key feature of diabetes and play an important role in initiating and propagating oxidative stress and inflammation that fuels both endothelial dysfunction and the eventual loss of pancreatic β-cells that is associated with late-stage type-2 diabetes. Our opinion is that AGEs represent the vital link between oxidative stress and inflammation and therefore represent an as-yet-untapped therapeutic opportunity, particularly if the AGE/RAGE interaction proves to be as important as some suggest.

C. Systemic hypertension

Systemic hypertension is a major risk factor for coronary heart disease (131), stroke (318), and death (9). The precise cause of hypertension is unknown but is likely to be multifactorial and to involve genetic predisposition, related primarily to the kidney (364) and responses to environmental stimuli (*e.g.*, obesity, high salt intake). Hypertension is associated with several neuroendocrine abnormalities, including activation of the renin–angiotensin system, sympathetic ner-

vous system, and increased expression of ET-1 (284, 337, 469). Release of Ang II is reported to stimulate transcription of pre-proendothelin-1, resulting in elevated ET-1 expression–a form of endothelial dysfunction. However, the link between Ang II and endothelial dysfunction is further exacerbated by its role in instigating oxidative stress, in part through upregulation of NOX enzymes, either directly (233) or *via* ET-1 (327). The precise role of oxidative stress in hypertension is not yet fully understood; ROS apparently induce MAP kinase activation (108), but their role in resistance vessel vasoconstriction (that might be anticipated on account of endothelial dysfunction) is not always found to be the case (108, 424). In clinical studies, endothelial dysfunction, as measured by vasodilator responses, appears to be a feature in hypertensive patients and a factor in the progression to overt cardiovascular disease. Several studies have shown impairment of endothelium-dependent vasodilatation in hypertensive patients (176, 252), and endothelial dysfunction has been demonstrated in patients at risk of developing hypertension even before hypertension occurs (252). It is likely that decreased bioavailablity of NO plays an important role in this phenomenon, given that NOS activity is reduced in patients with hypertension (284). Structural changes in the vessel wall also add to an overall decrease in vascular function and increased arterial stiffness (324).

Endothelial dysfunction is clearly associated with hypertension but, rather than being causal in the manifestation of the condition, it appears that it contributes to the progression of hypertension and the onset of atherosclerosis. Hypertension is a good example of a condition in which many different facets of "endothelial dysfunction" come together to contribute to the pathology, including ET-1, Ang II, NO, and EDHF.

D. Pulmonary hypertension

Pulmonary hypertension is a condition of elevated pulmonary arterial pressure that can lead to right ventricular hypertrophy and right heart failure if untreated. Endothelial cell proliferation and abnormal neovascularization are characteristic pathologic features in idiopathic pulmonary hypertension, but the triggers for these events are unknown. It is clear, however, that the processes involved are complex (50), and that an increase occurs in activated circulating endothelial cells of unknown origin (51), reduced expression of PGI_2, and an increase in expression of smooth muscle ET_B receptors in the lungs of patients with primary pulmonary hypertension. In addition, several other factors, such as thromboxane, vascular endothelial growth factor, NO, polyamines, and xanthine dehydrogenase (X-DH) have been implicated in the development and progression of primary pulmonary hypertension.

Pulmonary hypertension secondary to other disease states is widespread. Diseases that can cause pulmonary hypertension include hypoxic lung disease, chronic obstructive pulmonary disease, left heart failure, congenital heart disease, and HIV. Furthermore, a genetic component to the condition may be present, with mutations in the gene encoding bone morphogenetic protein receptor 2 (BMPR2) occurring in the majority of patients with familial pulmonary hypertension (297). Pulmonary hypertension is a complex, multifactorial condition involving vascular hypertrophy and abnormalities in the contraction and relaxation of pulmonary arteries, facilitated by endothelial dysfunction. Various mediators have been implicated, most notably IL-1, IL-6 (186), TNF-α (385), and vascular endothelial growth factor (VEGF) (432). However, evidence is accumulating in support of oxidative stress as a key factor underlying the cellular changes and endothelial dysfunction. Patients with primary and secondary pulmonary hypertension have increased plasma malondialdehyde (a marker of oxidative stress) (191) and reduced lung SOD expression (35). Ischemia has been shown to stimulate ROS production in pulmonary capillaries, mainly from endothelial cells (2); administration of antioxidants protects against the increase in pulmonary artery pressure (109), whereas SOD administration limits vasoconstrictor hypersensitivity (259). Increasing pulmonary blood pressure upregulates $p67^{phox}$ and $gp91^{phox}$ (259) in NOX (2), downregulates SOD (83), and increases endothelial levels of xanthine oxidase (166), causing impaired endothelium-dependent relaxation (397), probably through inactivation of NO (78). The footprint of resultant $ONOO^-$ (nitrotyrosine) has been found in the endothelium of both conduit and resistance pulmonary vessels (91); $ONOO^-$ also inactivates Mn-SOD, further promoting oxidative stress (474). Further to compound the issue, eNOS expression is reduced in the lungs of patients with pulmonary hypertension (139); eNOS-deficient mice exposed to hypoxia have exaggerated pulmonary hypertension (115), but those overexpressing the enzyme are resistant to the condition (329). ROS also stimulate hypoxia-inducible factor 1 (HIF-1) transcription factor, a key component in many of the long-term changes of chronic hypoxia. HIF-1 regulates the expression of VEGF under hypoxia, and high levels of HIF-1α have been found in proliferating endothelial cells of lung plexiform lesions in patients with pulmonary hypertension (375). HIF-1α–deficient mice exposed to prolonged hypoxia have reduced right ventricular hypertrophy and vascular remodeling. DPI and catalase inhibit HIF-1α, signifying that superoxide and H_2O_2 are linked to HIF-1α activation (149).

PGI_2 also helps to control pulmonary pressure. Patients with idiopathic pulmonary hypertension or HIV-associated pulmonary hypertension (431) have reduced PGI_2 synthase expression, which is likely to contribute to increased platelet aggregation. PGI_2 generation is reduced by hypoxia in pulmonary endothelial cells (15), and PGI_2 receptor-deficient mice are hypersensitive to chronic hypoxia (182).

A range of contractile mediators are also altered in pulmonary hypertension, further contributing to increased pulmonary pressure and remodeling. ET-1 is a potent vasoconstrictor and co-mitogen that is elevated in the plasma of pulmonary hypertensive patients (72) and animal models of the disease (249). mRNA of both ET_A- and ET_B-receptor subtypes is increased in animal models (249), although ET-1 vasodilatation through endothelial ET_B receptors is impaired (106). The proliferative effect of ET-1 appears to be mediated mainly through ET_A receptors (484), possibly through a pathway involving superoxide, as ET-1 stimulates superoxide generation in cultured pulmonary artery smooth muscle cells (460). ET-1 also increases pulmonary endothelial Ang II secretion (205), which in turn upregulates ET_A expression on smooth muscle cells (167) and stimulates superoxide production through NOX (155).

5-HT is another key vasoconstrictor and pulmonary smooth muscle cell mitogen that increases superoxide production *via* NOX, exacerbating vasoconstriction (258) and stimulating c-fos (382). 5-HT is elevated in the plasma of patients with primary pulmonary hypertension (175), possibly as a result of upregulation of angiopoietin-1, which stimulates pulmonary endothelial cells to produce and secrete 5-HT (407). Experimentally, 5-HT treatment during chronic hypoxia induces pulmonary hypertension, right ventricular remodeling, and mitogenesis in pulmonary vessels in rats (105), whereas right ventricular hypertrophy and vascular remodeling is less evident in 5-HT_{1B}–knockout mice (208). 5-HT_{2B}–receptor expression is increased in patients with pulmonary hypertension, and 5-HT_{2B}–receptor knockout hypoxic mice do not display increased pulmonary arterial pressure or lung remodeling.

E. Heart failure

Heart failure is defined as the condition that ensues as a result of insufficient cardiac output to meet demand, resulting in breathlessness and fatigue, which can be extremely debilitating in advanced cases. The most common cause of heart failure is ischemic heart disease as a result of coronary artery obstruction, resulting in chronic myocardial ischemia or myocardial infarction. However, other etiologies exist, including viral myocarditis, drug- or toxin-induced (anthracyclins, alcohol) disorders, or end-stage severe valvular heart disease. Regardless of the initial causal etiology, chronic heart failure is a complex neurohormonal syndrome associated with activation of the renin–angiotensin system (128) and peripheral vasoconstriction (487). At first glance, endothelial dysfunction might not be expected to play a direct role in heart failure, and indeed much of the emphasis with respect to oxidative stress in heart failure is focused on the heart itself (140).

However, considerable evidence indicates that increased peripheral vascular resistance associated with heart failure is partly due to endothelial dysfunction (373), although hyperactivity of the renin–angiotensin system and the sympathetic nervous system also is heavily involved in the pathogenesis of the condition. Heart failure is a good example of a disease in which endothelial dysfunction *per se* has many facets. Both animal and human studies have shown that vasodilatation in response to endothelium-dependent vasodilators is blunted in heart failure, but the effect is likely due both to reduced eNOS expression (262) and to increased inactivation of NO by ROS (288), which might in turn result in cytotoxic effects in endothelial cells through the actions of $ONOO^-$. Inflammation is most likely central to both eNOS regulation and ROS production in this setting, with enhanced neutrophil activation and cytokine release featuring in the disease. However, it is also important to note that clear evidence exists for enhanced release of ET-1 in heart failure, and that this and the renin–angiotensin system are also likely to play important roles in the cycle of events that fuels the progression of the disease. As mentioned earlier, Ang II itself promotes oxidative stress through NOX expression and activity.

Some data suggest that endothelial dysfunction in both the coronary (309) and peripheral (172, 204) circulation is associated with a poorer prognosis in patients with CHF, although age and renal function may have confounded these results to some degree (172). Whether the endothelial dysfunction is a primary or secondary feature of heart failure is unknown, although it appears to be a feature of both ischemic and nonischemic CHF, suggesting that it may be an important secondary feature. Regardless of its contributory role to the development of congestive heart failure, impaired peripheral endothelial function in these patients predicts a worse outcome and may yet become a useful clinical tool to identify patients at higher risk and allow targeted intervention.

F. Ischemia–reperfusion injury

Ischemia–reperfusion (I/R) injury is a well-recognized phenomenon that is primarily caused by oxidative stress after reoxygenation of ischemic tissues caused, for example, during organ transplant, bypass surgery, or recanalization after myocardial infarction. I/R is characterized by activation of inflammatory cells [particularly neutrophils (263, 446, 465)] and reduced viability of endothelial cells, which is both initiated and exacerbated by generation of ROS (199) in the oxidative burst after reoxygenation (Fig. 14). It is widely acknowledged that the clinical manifestations of I/R are primarily underpinned by the effects of inflammatory cell activation to release inflammatory cytokines (488), ROS, and $ONOO^-$ (255), combined with the loss of endothelial cells that normally release protective endothelium-derived relaxing factors [for review, see (127)]. The resulting vasoconstriction of the microcirculation, together with an increased tendency for platelet aggregation, monocyte adhesion, and leukocyte activation, is a critical limitation to organ survival caused, at least in part, by reduced endothelial NO synthesis and increased inactivation of NO by oxygen-derived free radicals (357). Furthermore, release of inflammatory cytokines by neutrophils and other inflammatory cells triggers chronic inflammation that is often associated with I/R injury. In the coronary circulation, some evidence suggests that the microvasculature is more susceptible to endothelial dysfunction than are the epicardial coronary arteries (340).

Several strategies are used to try to mitigate against reperfusion injury. Organs for transplant are cooled as quickly as possible after blood-flow cessation by perfusing with an organ-preservation solution, which often contains allopurinol to inhibit xanthine oxidase activity, together with glutathione by way of antioxidant and a glucocorticoid to help prevent inflammation (*e.g.*, University of Wisconsin solution). Cooling the organ slows respiration and helps to prevent the oxidative burst on reperfusion, but recent advances suggest that donor blood might be oxygenated and perfused throughout the organ-storage phase of the procedure, thus preventing the ischemic episode and avoiding the issue of ischemia–reperfusion altogether. However, this technique is likely to be of merit only in transplants from a living donor, as autologous blood is likely to be best fit for the purpose. Endothelial function might be better protected by other additives to cold-storage solutions: glutathione is not the ideal antioxidant to include on the basis that it does not penetrate membranes easily and is, therefore, likely to remain in the extracellular environment and remote from the intracellular source of most of the ROS. Indeed, our recent research indicated that glutathione (3 m*M*) in the extracellular environ-

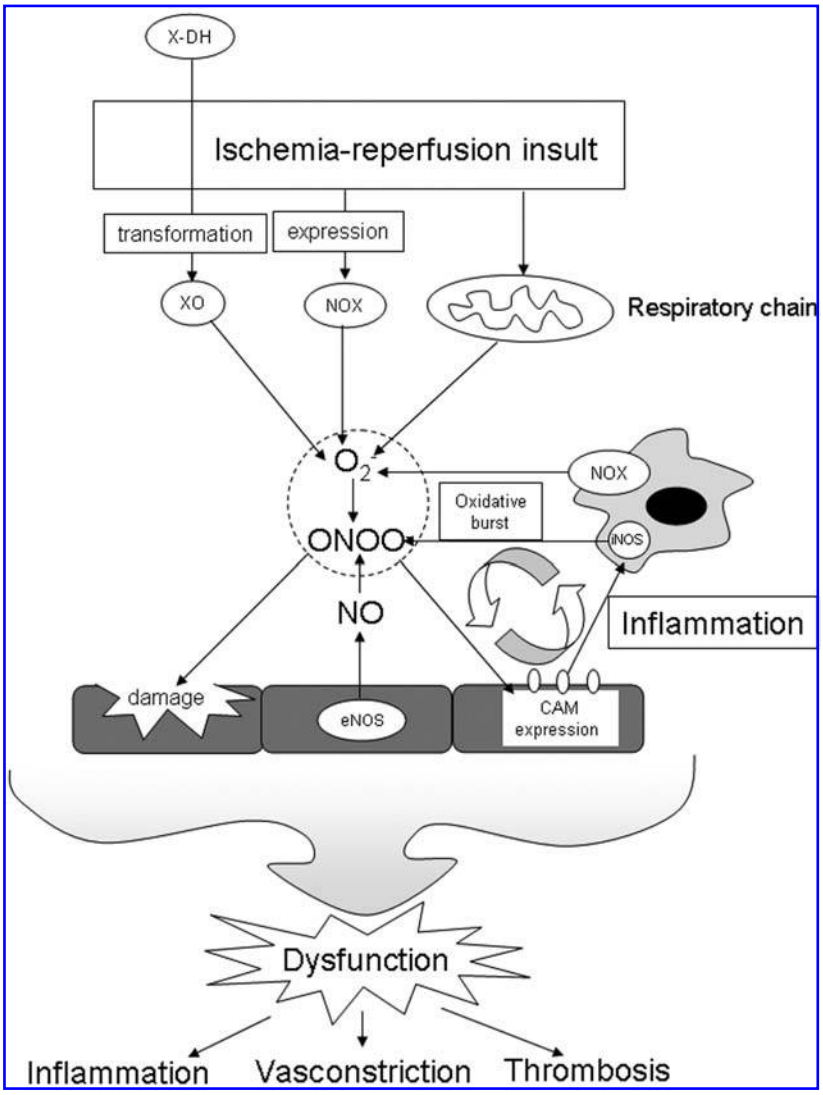

FIG. 14. Ischemia–reperfusion injury: role of reactive oxygen and reactive nitrogen species in mediating the inflammatory response and endothelial dysfunction. X-DH, xanthine dehydrogenase; XC, xanthine oxidase; NOX, NAD(P)H oxidase; endothelial eNOS, nitric oxide synthase.

ment causes a paradoxic augmentation of endothelial dysfunction and depressed endothelial survival (411).

An alternative strategy that was first used in the heart and has since been applied to other organs, in advance of ischemia during surgery, is known as preconditioning. This involves exposing the heart to a brief ischemic episode (a few minutes) before the main ischemic period. Much of the benefit of this approach affects the cardiac myocytes, which apparently benefit from phosphorylation events in the mitochondria, but recently, attention has also focused on potential benefits in the endothelial cells, most notably *via* inhibition of ET-1–mediated activation of XO and NOX (99) and *via* AKt-mediated survival pathways (492).

X. The Future of Endothelial Function Measurement

A. Endothelial function in predisease

Perhaps the most valuable application for endothelial-function measurement is not the demonstration of endothelial dysfunction in patients with established disease, but the identification of asymptomatic patients with risk factors who demonstrate endothelial dysfunction (*i.e.*, in predisease states). This might include patients with hypercholesterolemia, hypertension (293), or a family history of vascular disease, but who do not (yet) fall into a high-cardiovascular-risk category. It might be envisaged in the near future that the measurement of endothelial dysfunction could identify patients at an early stage for medical or lifestyle interventions.

B. Assessing impact of therapies in individuals by using endothelial function

The rationale for "evidence-based medicine" is that population-based outcomes can be extrapolated to the individual patient. In some cardiovascular therapies, we may measure effect (*e.g.*, blood pressure, renal function), but the role of the vasculature or neuroendocrine system is not monitored, and thus, a proportion of patients may receive therapies that are either insufficient or ineffective in that individual. Noninvasive measurement of vascular function may identify responders to drug therapy, or allow titration of therapy based on effect, thus facilitating tailored cardiovascular therapies.

Improvements in vascular function have been documented with angiotensin blockade in patients with hypertension (177, 245, 433), coronary artery disease (6, 268) and heart failure (194). Similar studies investigating the effect of HMG-CoA inhibitors (statins) are conflicting: some studies have demonstrated improvements in endothelial function with statins (3, 64, 100), whereas others have shown no effect (22, 440); differences in study techniques may explain some of these conflicting results.

C. Prognostic value of endothelial function measurement

Several studies have shown an association between measured endothelial dysfunction and outcome in patients with heart failure (172), coronary disease in both the coronary (161, 366, 408) and peripheral (173, 312) vasculature, hypertension (333), and even in "healthy" subjects. The clinical importance of this is uncertain, and the use of invasive techniques to refine prognosis alone may not be clinically useful.

In summary, several techniques are used for measuring endothelial function, but none is ideally suited for clinical practice because they are either too invasive or too expensive (coronary studies and forearm plethysmography) or are difficult to standardize (brachial artery flow-mediated dilatation). Simpler techniques will have to be developed and assessed before measuring endothelial function can be used as a reproducible diagnostic tool.

XI. Prevention of Endothelial Dysfunction

Prevention of endothelial dysfunction is clearly of great importance in avoiding or ameliorating the development of some aspects of vascular disease, although, given that it is a natural process associated with aging, it might not be truly "preventable." An appropriate healthful diet, avoiding certain "toxic" substances (*e.g.*, cigarette smoke), taking regular exercise, and maintaining a health body weight clearly all have important health benefits that include the maintenance of a healthy endothelium. The greatest challenge that faces public health physicians is how to encourage populations to adopt these measures. In general, population-based interventions have been disappointingly ineffective because convincing the population as a whole to undertake fundamental changes in behavior is difficult and extremely costly. Thus, targeted intervention in "motivated" high-risk patient groups has been adopted with some success (*e.g.*, cardiac rehabilitation programs after myocardial infarction). Nevertheless, population-based and fiscal interventions such as increasing the tax on tobacco or banning cigarette smoking in public places may have wider-reaching benefits to the whole population. Early reports regarding the ban on smoking in public places in Scotland indicated a 17% reduction in MI-related hospital admissions since the ban came into effect in 2006, compared with a 3% reduction per annum in years preceding the ban.

XII. Therapies for Endothelial Dysfunction

As discussed at length earlier, endothelial dysfunction can be manifest as a triggering event for disease processes (*i.e.*, a contributory factor to cause of disease), but can equally be a later manifestation of disease progression that worsens the impact (*i.e.*, an effect of disease that is nevertheless an important facet in severity). The multifactorial nature of cardiovascular diseases necessarily means that treating a single aspect is unlikely to have a significant impact on outcome. This concept is highlighted by the fact that diseases like heart failure and hypertension are treated with combinations of drugs (*e.g.*, angiotensin converting enzyme inhibitors/β-blockers, calcium channel blockers), some of which themselves have multiple effects (*e.g.*, ACE inhibitors reduce blood volume, reduce peripheral vascular resistance, have long-term benefits in terms of cardiac and vascular remodelling, and might be likely to have an impact on oxidative stress by reducing NOX activity). Endothelial dysfunction is not an innocent bystander in any of the disease processes detailed and therefore represents a legitimate target for drugs that would act in concert with existing therapies to reduce both the cause and effect of disease.

A. Current: statins (pleiotropic effects)

Given the wealth of evidence supporting a role for endothelial dysfunction in one form or another in a range of cardiovascular conditions, including atherosclerotic disease, it is perhaps surprising that few of the ongoing major drug trials in atherosclerosis specifically target the endothelium, although arguably, most might have an indirect impact on the endothelium (326). The same is true for the existing therapy for atherosclerosis and the related clinical conditions: statins. This group of compounds inhibits 3-hydrox-3-methylglutaryl coenzyme A (HMG-CoA), which is involved in the endogenous synthesis of cholesterol. Whereas lipid-lowering is the primary target of statins, it has since been established that they also mediate a number of so-called "pleiotropic effects." Improved endothelial function (232) is one of these effects, alongside antioxidant, antiplatelet, and antiinflammatory actions. Amelioration of endothelial dysfunction is mediated by a number of processes, including interference with pathways associated with oxidative stress [for review, see (271)]. As yet, it is unclear just how much impact the pleiotropic effects have on clinical outcome over and above the primary benefit of lipid-lowering, but the beneficial effects of statins on endothelial function at least raise the possibility of this feature of cardiovascular disease being a legitimate therapeutic target.

To date, endothelial dysfunction has not been recognized as a primary therapeutic target in other cardiovascular diseases (*e.g.*, hypertension and heart failure), in which conventional therapies concentrate on reducing volume overload through diuresis (diuretics) and renin–angiotensin system–mediated effects either directly (ACE inhibitors, angiotensin-receptor antagonists) or indirectly *via* β-adrenoceptor antagonism (β-blockers), although direct vasodilators (calcium channel blockers, GTN) also have some merit in this arena. It is not yet clear whether any of these conventional treatments actually has an impact on improving endothelial function; for example, the evidence relating to the effects of antihypertensive drugs on endothelial function is conflicting (112, 303). Given the increased interest in the profile of endothelial dysfunction in these two conditions over the last few years, it is possible that drugs specifically targeted at the endothelium might evolve, although the market is already congested.

B. Possible future treatments for eNOS dysfunction

A number of different strategies might be adopted to overcome the various forms of eNOS dysfunction. Ever since L-arginine was discovered as the precursor of NO, its potential as a therapeutic agent to boost NO production has been predicted. However, the obvious flaw in the hypothesis is that, for supplementation of L-arginine to be effective, endothelial L-arginine levels would have to be depleted to such an extent for the enzyme to malfunction: this is particularly unlikely in the case of eNOS, in which enzyme activity is typically low and therefore only requires low levels of L-arginine. However, unlikely as it is that L-arginine is deficient in endothelial cells, many studies have shown a benefit of L-arginine supplementation in terms of endothelial function in a range of cardiovascular disease states (242). A number of theories have been developed to explain this "arginine paradox," including proposals that high arginine is simply acting in an antioxidant capacity. However, the most plausible explanation has evolved since the recent discovery of asymmetric dimethyl-L-arginine (ADMA), an endogenous L-arginine analogue that can inhibit NOS [for review, see (28)] and contribute to oxidative stress [reviewed in (29, 239)], possibly through invoking an imbalance in NO/O_2^- generation from NOS itself (61). By the law of mass action, increased L-arginine levels might act effectively to compete with ADMA at both the Y^+ transporter responsible for its uptake into cells and at NOS itself (Fig. 7). Conflicting results regarding the therapeutic benefit of L-arginine (or otherwise) mean that the evidence is unclear as to whether, or to what extent, L-arginine supplements might help in cardiovascular disease. In the opinion of the authors of this review, any benefits that might accrue from L-arginine supplementation are not likely due to simply replenishing a shortfall in eNOS substrate. Benefit might be achieved, however, in cases in which ADMA is a factor or, perhaps, where dysfunctional iNOS is chronically expressed and is depleted of substrate on account of its rapid turnover. What is clear is that the interaction of L-arginine with NOS is dependent as much on the activity of the enzyme responsible for its conversion to ornithine, arginase (102), and on the synthesis and metabolic pathways (*e.g.*, dimethylarginine dimethylaminohydrolase; DDAH) relating to ADMA (23) as it is on the plasma concentration of L-arginine (Fig. 7).

Although the precise role of BH_4 in NOS is still not completely clear, its importance in preventing NOS dysfunction is without question (4, 67, 213). It follows that agents that might act to increase BH_4 bioavailability in endothelial cells would act to improve eNOS function in those conditions in which BH_4 deficiency is central to the dysfunction experienced. Once again, *in vitro* studies using BH_4 supplementation have shown promise in several cardiovascular disease models. However, the therapeutic potential of this agent is limited by its poor oral absorption and bioavailability (120), and a number of strategies are in development to deliver bioactive BH_4 analogues (350, 442) for this purpose. Alternatively, as we learn more about the synthesis and metabolism of BH_4 [Fig. 6; see (294) for review], new therapeutic avenues might emerge that could exploit these pathways to maintain or replenish BH_4 in individuals with depleted levels. One such avenue is already emerging: homocysteine has long been recognized to have an impact on endothelial function, but the reason for its detrimental effects was not obvious at the outset. It has emerged, however, that homocysteine reduces the bioavailability of BH_4, either *via* direct interaction or through inhibition of one of the enzymes [sepiapterin reductase (SR)] responsible for its synthesis (Fig. 7) (294). Reduction of homocysteine levels through administration of folate might, somewhat surprisingly, increase BH_4 levels.

C. Nitric oxide and carbon monoxide donor drugs

Given that depressed bioavailability of NO is a prominent cause of endothelial dysfunction, it follows that replacement of NO from exogenous sources could be of great benefit in ameliorating disease progression. Organic nitrates have long been used in symptomatic treatment of angina. Strangely, however, most studies indicate that organic nitrates do not actually reduce mortality in patients. Since the discovery of NO as a crucial mediator in the cardiovascular system during the 1980s, it was widely anticipated that a wide range of NO-donor drugs would evolve for use in cardiovascular disease. No new drugs in this class have been licensed (283, 289), prompting speculation that this is a flawed strategy. Part of the issue surrounds the inability to target NO suitably to areas of endothelial dysfunction without incurring overwhelming vasodilatation and hypotension. Some strategies are beginning to evolve for targeted NO delivery; inhaled NO is now routinely used to help alleviate breathing difficulties in neonates and has been proposed as a means of limiting the effects of pulmonary hypertension (189). In addition, a number of new donor entities are in development that effect targeted NO delivery, primarily through incorporation in materials used in stents and other devices that might come into contact with the vascular wall and contribute to endothelial dysfunction (289). Despite the setbacks in NO-based drug therapies, hopes remain high that novel agents might yet supersede organic nitrates in protection from, or reversal of, endothelial dysfunction.

Research into a therapeutic role for CO is still in its infancy, but several CO-donor drugs have been developed (379) and have been shown to have an impact in ischemia–reperfusion injury and transplant [reviewed in (306)]. Inhaled CO has also been shown to have some merit in reducing arterial thrombus formation (429).

D. Phosphodiesterase inhibitors and activators of guanylate cyclase

Downstream modulators of the NO/guanylate cyclase pathway are receiving considerable attention. Sildenafil (Viagra) is a phosphodiesterase V (PDE V) inhibitor that acts to depress cGMP breakdown by this enzyme. It was originally developed in the wake of the discovery of the NO/sGC pathway with a view to its use in cardiovascular conditions. Thanks to the prevalence of PDE V in the corpus cavernosal tissue of the penis, its development was rapidly redirected to the lucrative market of sexual dysfunction. However, attention is now reverting to the possible benefits of agents like sildenafil that act to protect cGMP or non-NO activators of sGC [*e.g.*, Bay41-2272 (393)] as therapeutic agents for cardiovascular disease. Sildenafil has potentially important effects on vascular reactivity through improved endothelial function in the peripheral vasculature of otherwise healthy

cigarette smokers (215), as well as in patients with chronic heart failure (286) and in the coronary circulation of patients with coronary heart disease (160), although other studies show no effect on endothelial function (354). Nevertheless, treatment with sildenafil has shown promising benefits in patients with chronic heart failure (203) and pulmonary hypertension (286), alone or in combination with inhaled NO (396), or the PGI_2 analogue, iloprost (463).

E. Prostanoids

Prostanoid therapies have failed to make the anticipated impact on cardiovascular disease. Pulmonary hypertension is the most evident cardiovascular condition in which prostanoid vasodilators, such as PGI_2 and iloprost (463), have come to the fore as credible therapeutic agents.

F. Antioxidant therapies

Given the key role of oxidative stress in many cardiovascular conditions, use of broad-spectrum antioxidants might be expected to be effective therapeutics. The *in vitro* data are very encouraging for a number of natural antioxidants, including vitamins A, C, and E, thiols [*e.g.*, N-acetylcysteine (NAC)], and plant-derived polyphenols (*e.g.*, flavonoids). The promise of such antioxidants is further supported in clinical studies using standard measures of endothelial function (157), but, unfortunately, the large clinical trials so far conducted with vitamin A (174, 224, 229, 352, 419) and vitamin C (26) have shown no clear beneficial effects. Vitamin E at least showed some benefit in one trial (400), but others have failed to show a clinical benefit, even in high-risk patient populations (157, 310, 400, 482, 483). The reason(s) behind the failure of dietary antioxidant vitamins to have the expected beneficial effect, given the overwhelming data to support antioxidants from preclinical and small-scale clinical studies, is(are) not clear. Low doses of vitamin could explain some of the negative effects, but even in trials of higher doses, no positive effect was seen; the lack of effect of vitamins A, C, and E on cardiovascular outcomes appears to be a consistent finding in these well-conducted large-scale clinical trials. However, the effects of antioxidant vitamins may be subtle, and investigating crude outcome measures such as death and cardiovascular events may miss subtle beneficial effects on endothelial function. Equally, the benefits might be seen only with prolonged (life-long) treatment. Furthermore, dietary vitamins, although attractive because of their relatively low cost and high tolerability, might not be the most suitable antioxidants for this target. Some evidence suggests that antioxidant supplements do not necessarily mimic the effects of whole-fruit/vegetable dietary interventions, perhaps suggesting that other fruit- and vegetable-derived agents are important or that either additive or synergistic effects of fruit and vegetable-derived nutrients may accrue [for reviews, see (311,419)]. Perhaps, therefore, we are seeking a "silver bullet" that does not exist and, rather than looking for an antioxidant supplement "quick fix" for our inherently unhealthy lifestyle, we should default to achieving overall dietary improvements that might reduce oxidative stress in the first place. What is clear to date is that insufficient evidence exists to support the hypothesis that oral vitamin supplementation is protective against cardiovascular disease in well-nourished populations (*i.e.*, not deficient in these vitamins). However, it is worth remembering that, although dietary vitamins might not offer the whole answer, an enormous amount of work is ongoing to suggest that polyphenolic dietary antioxidants (*e.g.*, resveratrol in red wine and berries and catechin and epicatechin in chocolate) might yet prove beneficial (122, 201), whereas other means of enhancing endogenous antioxidant defenses (*e.g.*, gene transfer for increased expression of antioxidant enzymes) might also prove an effective therapeutic approach (see later).

G. Endothelin antagonists

Intensive research has been performed into the clinical development of several endothelin antagonists (345). However, to date, these efforts have been disappointing in most clinical conditions, with the exception of primary pulmonary hypertension. Both selective ET_A and combined $ET_{A/B}$ have been investigated, but the comparative effects of these in clinical trials remains unknown. The major issues with endothelin antagonists to date have been the lack of significant mortality benefits, elevation of hepatic transaminases in clinical studies, and the potential for teratogenicity.

Several endothelin antagonists have delivered important blood pressure–lowering effects in patients with systemic hypertension, although given the large number of drugs already available for this condition, they have not gained a clinical license. However, the combined $ET_{A/B}$ endothelin antagonist, bosentan, has proven clinical benefits in patients with primary pulmonary hypertension (20) and, because of the lack of alternatives, has gained a clinical license for use in these patients. To date the results in most clinical studies in acute and chronic heart failure have been disappointing (14). Furthermore, concerns about teratogenicity with endothelin antagonists are likely to limit their future clinical use. Studies of endothelin-converting enzyme (ECE) and combined neutral endopeptidase (NEP) inhibitors are continuing, but their future clinical usefulness is currently unknown.

H. Gene therapies

Endothelial dysfunction is a prime target for gene based therapies, not least because of the accessibility of endothelial cells to blood-borne agents. Gene targeting can be enhanced by using endothelial cell–specific promoter sequences to drive expression of transgenes delivered in adenoviral vectors; flt-1 and ICAM-2 are among the most specific promoters used to date (316). Alternative methods of improving specificity of adenoviral vector–derived gene transfer for endothelial cells include replacement of retrovirus long terminal repeat with regulatory sequences from human promoters of endothelium-specific proteins (*e.g.*, preproendothelin-1, von Willebrand factor) or genetically altering the vector itself to target endothelial cells (192, 276, 346). Adenoviral vectors are generally regarded to be more efficient means of transfer than delivery of naked plasmids or those in cationic liposomes (285). A number of cellular targets exist for gene therapy–mediated upregulation of protein expression, most notable of which are eNOS (1, 68), enzymes that contribute to antioxidant defences [*e.g.*, SOD (93, 119, 236, 250)], enzymes involved in BH_4 modulation (4, 5, 59), or fibrinolytic proteins (t-PA). An alternative strategy that has been used successfully in animal models of myo-

cardial infarction is to prevent the pro-inflammatory, pro-oxidant activation of endothelial cells through activation of NF-κB (37, 365). This can be achieved by delivery of a "decoy" oligonucleotide bearing the consensus binding sequence of NF-κB (296). The current limitations to these techniques surround the effective delivery of sufficient gene copies by a practical means for human use, but the concept holds considerable promise if these issues can be overcome.

I. Endothelial cell–based therapies

Another approach involves use of autologous endothelial progenitor cells (EPCs). These bone marrow–derived cells express many endothelial cell markers and are freely circulating in humans. Their isolation and culture from blood samples is fairly straightforward, and they are highly amenable to genetic modification through adenoviral vector–mediated gene transfer (13, 156). The concept is to isolate, culture, and modify endothelial cells from patients with diseases associated with endothelial injury before reinjecting them, in the hope that they will effect a repair to areas where the endothelium is damaged. This approach could be particularly useful in aiding re-endothelialization after interventional procedures like angioplasty or stenting and has also shown promise in formation of new vessels in ischemic tissue. The number of circulating EPCs is typically very low and is further depressed in a range of cardiovascular diseases (103), but they appear to be mobilized in response to vascular endothelial growth factor (VEGF) and granulocyte colony-stimulating factor (G-CSF). Therapeutic elevation of circulating EPC numbers might also be an alternative strategy to helping endothelial repair. As with gene therapy, the use of EPCs for treatment of specific conditions associated with endothelial injury shows promise, even in small-scale clinical studies using unmodified autologous EPCs [for review, see (103)].

XIII. CONCLUSIONS

In view of the very wide range of functions carried out by the endothelium, it follows that "endothelial dysfunction" is a term that applies to an equally wide range of endothelium-related aspects that might be considered abnormal. In the literature, it is usual for only a single parameter to be assessed as a measure of endothelial dysfunction. Vascular response to an endothelium-dependent vasodilator (*e.g.*, ACh or bradykinin) is by far the most common approach to assessment of endothelial dysfunction *in vitro*, *in vivo*, and in clinical studies, but this will identify only one form of the phenomenon. Equally, such an approach will not provide any information as to the reason for reduced endothelial activity (*e.g.*, reduced NOS expression, dysfunctional NOS, lack of BH_4, increased ADMA, increased oxidative stress, increased ET-1, reduced prostaglandin synthesis, or EDHF activity). In animal models, an initial observation of endothelial dysfunction can be followed up by mechanistic experiments *in vitro* and in knockout models to dissect the likely cause(s) of the effect; deeper exploration in clinical studies is considerably more difficult.

Endothelial dysfunction has emerged as a contributory factor in a wide range of cardiovascular diseases. However, the means by which dysfunction is defined and measured vary greatly between researchers and specific diseases. Thus, endothelial dysfunction in atherosclerosis is driven by different processes, with different measurable outcomes than that in, for example, hypertension. Whereas the term is unifying in the sense that it identifies the endothelium as a central player in many disease processes, it does not identify a single unifying process that underlies different cardiovascular disease and, therefore, does not point to a single therapeutic strategy that might encompass different diseases. It is essential, therefore, for researchers first to identify the type of endothelial dysfunction that applies to the disease of interest (*e.g.*, is the endothelium physically removed or injured? Is NOS dysfunctional? Is endothelin upregulated? Is oxidative stress a factor? Does inflammation play a role?), before deciding what therapeutic approach might be beneficial. That said, now an enormous array of therapeutic options is available to target each of the specific factors that might combine to constitute endothelial dysfunction. Both pharmaceutic and neutraceutic agents are under intense scrutiny in a drive to combat oxidative stress or to supplement or replace NO, or both, whereas gene therapy and EPC supplementation are perhaps therapeutic strategies to watch in the future. However, the diseases in which endothelial dysfunction contributes are all multifactorial and, although therapies that target this facet of disease progression might work well as an adjunct to conventional therapies, it seems unlikely that they represent a "cure" in their own right. In our opinion, endothelial dysfunction is a classic example of the "prevention is better than cure" adage, in that the classic lifestyle changes that are advocated for health (*e.g.*, exercise, good diet, smoking cessation, and weight management) are bound to have a significant and profound impact on endothelial function. Moreover, the earlier lifestyle changes are implemented, the more likely the benefit in terms of limiting the endothelial dysfunction that is recognized as a key early event in atherogenesis. Given that dysfunction is a natural aging process, it seems unlikely that we can altogether prevent it. However, it is within each individual's power to limit the damaging effects though lifestyle improvements.

ABBREVIATIONS

AA, Arachidonic acid; Ach, acetylcholine; ADMA, asymmetric dimethyl-L-arginine; Ang II, angiotensin II; AC, adenylate cyclase; AT_1, angiotensin receptor; BH_4, tetrahydrobiopterin; BK, bradykinin; cAMP, cyclic adenosine monophosphate; CB, cannabinoid receptor; cGMP, cyclic guanosine monophosphate; CO, carbon monoxide; COX, cyclooxygenase; CYP, cytochrome P450; DHFR, dihydrofolate reductase; EETs, epoxyeicosatrienoic acids; ET-1, endothelin 1; $ET_{A/B}$, endothelin A and B receptors; EDHF, endothelium-derived hyperpolarizng factor; GCL, glutamate-cysteine ligase; GPx, glutathione peroxidase; GPCR, G protein–coupled receptor; GR, glutathione reductase; GS, glutathione synthase; GSH, glutathione; GSSG, glutathione (oxidized form); GTP, guanosine triphosphate; GTPCH, GTP cyclohydrolase; HCys, homocyst(e)ine; HO, heme oxygenase; NO, nitric oxide; NOS, nitric oxide synthase; NOX, NAD(P)H oxidase; PGI_2, prostacyclin; PGIS, prostaglandin I_2 synthase; PGR, prostaglandin receptor; PKs, protein kinases; PLA_2, phopholipase A_2; PPAR-γ,_peroxisome proliferator–activated receptor-γ; PTPS, 6-pyruvoyltetrahydrobiopterin synthase; ROS, reactive oxygen species; sGC, soluble guanylate

cyclase; SOD, superoxide dismutase; SR, sepiapterin reductase; tPA, tissue plasminogen activator; VGCC, voltage-gated Ca^{2+} channel; X-DH, xanthine dehydrogenase; XO, xanthine oxidase.

References

1. Alexander MY, Brosnan MJ, Hamilton CA, Fennell JP, Beattie EC, Jardine E, Heistad DD, and Dominiczak AF. Gene transfer of endothelial nitric oxide synthase but not Cu/Zn superoxide dismutase restores nitric oxide availability in the SHRSP. *Cardiovasc Res* 47: 609–617, 2000.
2. Al-Mehdi AB, Zhao G, Dodia C, Tozawa K, Costa KVM, Ross C, Blecha F, Dinauer M, and Fisher AB. Endothelial NADPH oxidase as the source of oxidants in lungs exposed to ischemia or high K+. *Circ Res* 83: 730–737, 1998.
3. Alonso R, Mata P, De Andres R, Villacastin BP, Martinez-Gonzalez J, and Badimon L. Sustained long-term improvement of arterial endothelial function in heterozygous familial hypercholesterolemia patients treated with simvastatin. *Atherosclerosis* 157: 423–429, 2001.
4. Alp NJ and Channon KM. Regulation of endothelial nitric oxide synthase by tetrahydrobiopterin in vascular disease. *Arterioscler Thromb Vasc Biol* 24: 413–420, 2004.
5. Alp NJ, Mussa S, Khoo J, Cai S, Guzik T, Jefferson A, Goh N, Rockett KA, and Channon KM. Tetrahydrobiopterin-dependent preservation of nitric oxide-mediated endothelial function in diabetes by targeted transgenic GTP-cyclohydrolase I overexpression. *J Clin Invest* 112: 725–735, 2003.
6. Anderson TJ, Elstein E, Haber H, and Charbonneau F. Comparative study of ACE-inhibition, angiotensin II antagonism, and calcium channel blockade on flow-mediated vasodilation in patients with coronary disease (BANFF study). *J Am Coll Cardiol* 35: 60–66, 2000.
7. Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, Lieberman EH, Ganz P, Creager MA, and Yeung AC. Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol* 26: 1235–1241, 1995.
8. Angus JA and Wright CE. Techniques to study the pharmacodynamics of isolated large and small blood vessels. *J Pharmacol Toxicol Methods* 44: 395–407, 2000.
9. Antikainen R, Jousilahti P, and Tuomilehto J. Systolic blood pressure, isolated systolic hypertension and risk of coronary heart disease, strokes, cardiovascular disease and all-cause mortality in the middle-aged population. *J Hypertens* 16: 577–583, 1998.
10. Araki N, Higashi T, Mori T, Shibayama R, Kawabe Y, Kodama T, Takahashi K, Shichiri M, and Horiuchi S. Macrophage scavenger receptor mediates the endocytic uptake and degradation of advanced glycation end products of the Maillard reaction. *Eur J Biochem* 230: 408–415, 1995.
11. Arbustini E, Dal Bello B, Morbini P, Burke AP, Bocciarelli M, Specchia G, and Virmani R. Plaque erosion is a major substrate for coronary thrombosis in acute myocardial infarction. *Heart* 82: 269–272, 1999.
12. Armstrong EJ, Morrow DA, and Sabatine MS. Inflammatory biomarkers in acute coronary syndromes, part II: acute-phase reactants and biomarkers of endothelial cell activation. *Circulation* 113: e152–e155, 2006.
13. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, and Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 275: 964–967, 1997.
14. Attina T, Camidge R, Newby DE, and Webb DJ. Endothelin antagonism in pulmonary hypertension, heart failure, and beyond. *Heart* 91: 825–831, 2005.
15. Badesch DB, Orton EC, Zapp LM, Westcott JY, Hester J, Voelkel NF, and Stenmark KR. Decreased arterial wall prostaglandin production in neonatal calves with severe chronic pulmonary hypertension. *Am J Resp Cell Mol Biol* 1: 489–498, 1989.
16. Banes-Berceli AK, Ogobi S, Tawfik A, Patel B, Shirley A, Pollock DM, Fulton D, and Marrero MB. Endothelin-1 activation of JAK2 in vascular smooth muscle cells involves NAD(P)H oxidase-derived reactive oxygen species. *Vasc Pharmacol* 43: 310–319, 2005.
17. Barnes PJ and Karin M. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 336: 1066–1071, 1997.
18. Barton M, Cosentino F, Brandes RP, Moreau P, Shaw S, and Luscher TF. Anatomic heterogeneity of vascular aging: role of nitric oxide and endothelin. Hypertension 30: 817–824, 1997.
19. Basuroy S, Bhattacharya S, Tcheranova D, Qu Y, Regan RF, Leffler CW, and Parfenova H. HO-2 provides endogenous protection against oxidative stress and apoptosis caused by TNF-alpha in cerebral vascular endothelial cells. *Am J Physiol Cell Physiol* 291: C897–C908, 2006.
20. Bauer M, Wilkens H, Langer F, Schneider SO, Lausberg H, and Schafers HJ. Selective upregulation of endothelin B receptor gene expression in severe pulmonary hypertension. *Circulation* 105: 1034–1036, 2002.
21. Beckman KB and Ames BN. The free radical theory of aging matures. *Physiol Rev* 78: 547–581, 1998.
22. Beishuizen ED, Tamsma JT, Jukema JW, van de Ree MA, van der Vijver JC, Meinders AE, and Huisman MV. The effect of statin therapy on endothelial function in type 2 diabetes without manifest cardiovascular disease. *Diabetes Care* 28: 1668–1674, 2005.
23. Beltowski J and Kedra A. Asymmetric dimethylarginine (ADMA) as a target for pharmacotherapy. *Pharmacol Rep* 58: 159–178, 2006.
24. Berne RM. The role of adenosine in the regulation of coronary blood flow. *Circ Res* 47: 807–813, 1980.
25. Bierhaus A, Chevion S, Chevion M, Hofmann M, Quehenberger P, Illmer T, Luther T, Berentshtein E, Tritschler H, Muller M, Wahl P, Ziegler R, and Nawroth PP. Advanced glycation end product-induced activation of NF-kappaB is suppressed by alpha-lipoic acid in cultured endothelial cells. *Diabetes* 46: 1481–1490, 1997.
26. Blot WJ, Li JY, Taylor PR, Guo W, Dawsey S, Wang GQ, Yang CS, Zheng SF, Gail M, Li GY, Yu Y, Liu B, Tangrea J, Sun YH, Liu F, Fraumeni JF, Zhang YH Jr, and Li B. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence and disease-specific mortality in the general population. *J Natl Cancer Inst* 85: 1483–1492, 1993.
27. Blows LJ and Redwood SR. The pressure wire in practice. *Heart* 93: 419–422, 2007.
28. Boger RH. Asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, explains the "L-arginine paradox" and acts as a novel cardiovascular risk factor. *J Nutr* 134: 2842S–2847S; discussion 2853S, 2004.
29. Boger RH, Vallance P, and Cooke JP. Asymmetric dimethylarginine (ADMA): a key regulator of nitric oxide synthase. *Atherosclerosis Suppl* 4: 1–3, 2003.
30. Bollinger A, Hoffmann U, and Franzeck UK. Microvascular changes in arterial occlusive disease: target for pharmacotherapy. *Vasc Med* 1: 50–54, 1996.

31. Boo YC and Jo H. Flow-dependent regulation of endothelial nitric oxide synthase: role of protein kinases. *Am J Physiol Cell Physiol* 285: C499–C508, 2003.
32. Botti H, Trostchansky A, Batthyany C, and Rubbo H. Reactivity of peroxynitrite and nitric oxide with LDL. *IUBMB Life* 57: 407–412, 2005.
33. Boulanger CM, Morrison KJ, and Vanhoutte PM. Mediation by M_3-muscarinic receptors of both endothelium-dependent contraction and relaxation to acetylcholine in the aorta of the spontaneously hypertensive rat. *Br J Pharmacol* 112: 519–524, 1994.
34. Bouloumie A, Schini-Kerth VB, and Busse R. Vascular endothelial growth factor up-regulates nitric oxide synthase in endothelial cells. *Cardiovasc Res* 41: 773–780, 1999.
35. Bowers R, Cool C, Murphy RC, Tuder RM, Hopken MW, Flores SC, and Voelkel NF. Oxidative stress in severe pulmonary hypertension. *Am J Respir Crit Care Med* 169: 764–769, 2004.
36. Bowie AG, Moynagh PN, and O'Neill LA. Lipid peroxidation is involved in the activation of NF-kappaB by tumor necrosis factor but not interleukin-1 in the human endothelial cell line ECV304: lack of involvement of H_2O_2 in NF-kappaB activation by either cytokine in both primary and transformed endothelial cells. *J Biol Chem* 272: 25941–25950, 1997.
37. Boyle EM Jr, Canty TG Jr, Morgan EN, Yun W, Pohlman TH, and Verrier ED. Treating myocardial ischemia-reperfusion injury by targeting endothelial cell transcription. *Ann Thorac Surg* 68: 1949–1953, 1999.
38. Brand K, Page S, Rogler G, Bartsch A, Brandl R, Knuechel R, Page M, Kaltschmidt C, Baeuerle PA, and Neumeier D. Activated transcription factor nuclear factor-kappa B is present in the atherosclerotic lesion. *J Clin Invest* 97: 1715–1722, 1996.
39. Brandes RP, Schmitz-Winnenthal FH, Feletou M, Godecke A, Huang PL, Vanhoutte PM, Fleming I, and Busse R. An endothelium-derived hyperpolarizing factor distinct from NO and prostacyclin is a major endothelium-dependent vasodilator in resistance vessels of wild-type and endothelial NO synthase knockout mice. *Proc Natl Acad Sci U S A* 97: 9747–9752, 2000.
40. Brandt R and Krantz S. Glycated albumin (Amadori product) induces activation of MAP kinases in monocyte-like MonoMac 6 cells. *Biochim Biophys Acta* 1760: 1749–1753, 2006.
41. Brandt R, Nawka M, Kellermann J, Salazar R, Becher D, and Krantz S. Nucleophosmin is a component of the fructoselysine-specific receptor in cell membranes of Mono Mac 6 and U937 monocyte-like cells. *Biochim Biophys Acta* 1670: 132–136, 2004.
42. Brilla CG, Rupp H, Funck R, and Maisch B. The renin-angiotensin-aldosterone system and myocardial collagen matrix remodelling in congestive heart failure. *Eur Heart J* 16: 107–109, 1995.
43. Brook RD, Brook JR, and Rajagopalan S. Air pollution: the "Heart" of the problem. *Curr Hypertens Rep* 5: 32–39, 2003.
44. Broten TP, Miyashiro JK, Moncada S, and Feigl EO. Role of endothelium-derived relaxing factor in parasympathetic coronary vasodilation. *Am J Physiol* 262: H1579–H1584, 1992.
45. Brouard S, Berberat PO, Tobiasch E, Seldon MP, Bach FH, and Soares MP. Heme oxygenase-1-derived carbon monoxide requires the activation of transcription factor NF-kappa B to protect endothelial cells from tumor necrosis factor-alpha-mediated apoptosis. *J Biol Chem* 277: 17950–17961, 2002.
46. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 414: 813–820, 2001.
47. Bryan NS, Fernandez BO, Bauer SM, Garcia-Saura MF, Milsom AB, Rassaf T, Maloney RE, Bharti A, Rodriguez J, and Feelisch M. Nitrite is a signaling molecule and regulator of gene expression in mammalian tissues. *Nat Chem Biol* 1: 290–297, 2005.
48. Bucala R, Makita Z, Koschinsky T, Cerami A, and Vlassara H. Lipid advanced glycosylation: pathway for lipid oxidation in vivo. *Proc Natl Acad Sci U S A* 90: 14: 6434–6438, 1993.
49. Bucala R, Tracey KJ, and Cerami A. Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilation in experimental diabetes. *J Clin Invest* 87: 432–438, 1991.
50. Budhiraja R, Tuder RM, and Hassoun PM. Endothelial dysfunction in pulmonary hypertension. *Circulation* 109: 159–165, 2004.
51. Bull TM, Golpon H, Hebbel RP, Solovey A, Cool CD, Tuder RM, Geraci MW, and Voelkel NF. Circulating endothelial cells in pulmonary hypertension. *Thromb Haemost* 90: 698–703, 2003.
52. Bunning P, Budek W, Escher R, and Schonherr E. Characteristics of angiotensin converting enzyme and its role in the metabolism of angiotensin I by endothelium. *J Cardiovasc Pharmacol* 8: S52–S57, 1986.
53. Burney S, Niles JC, Dedon PC, and Tannenbaum SR. DNA damage in deoxynucleosides and oligonucleotides treated with peroxynitrite. *Chem Res Toxicol* 12: 513–520, 1999.
54. Burnham MP, Bychkov R, Feletou M, Richards GR, Vanhoutte PM, Weston AH, and Edwards G. Characterization of an apamin-sensitive small-conductance Ca(2+)-activated K+ channel in porcine coronary artery endothelium: relevance to EDHF. *Br J Pharmacol* 135: 1133–1143, 2002.
55. Busse R and Fleming I. Pulsatile stretch and shear stress: physical stimuli determining the production of endothelium-derived relaxing factors. *J Vasc Res* 35: 73–84, 1998.
56. Busse R, Forstermann U, Matsuda H, and Pohl U. The role of prostaglandins in the endothelium-mediated vasodilatory response to hypoxia. *Pflugers Arch* 401: 77–83, 1984.
57. Bychkov R, Burnham MP, Richards GR, Edwards G, Weston AH, Feletou M, and Vanhoutte PM. Characterization of a charybdotoxin-sensitive intermediate conductance Ca2+-activated K+ channel in porcine coronary endothelium: relevance to EDHF. *Br J Pharmacol* 137: 1346–1354, 2002.
58. Cai H and Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res* 87: 840–844, 2000.
59. Cai S, Alp NJ, McDonald D, Smith I, Kay J, Canevari L, Heales S, and Channon KM. GTP cyclohydrolase I gene transfer augments intracellular tetrahydrobiopterin in human endothelial cells: effects on nitric oxide synthase activity, protein levels and dimerisation. *Cardiovasc Res* 55: 838–849, 2002.
60. Cardillo C, Campia U, Bryant MB, and Panza JA. Increased activity of endogenous endothelin in patients with type II diabetes mellitus. *Circulation* 106: 1783–1787, 2002.
61. Cardounel AJ, Xia Y, and Zweier JL. 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* 280: 7540–7549, 2005.

62. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, and Deanfield JE. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 340: 1111–1115, 1992.
63. Ceriello A and Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. *Arterioscler Thromb Vasc Biol* 24: 816–823, 2004.
64. Ceriello A, Taboga C, Tonutti L, Quagliaro L, Piconi L, Bais B, Da Ros R, and Motz E. Evidence for an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial dysfunction and oxidative stress generation: effects of short- and long-term simvastatin treatment. *Circulation* 106: 1211–1218, 2002.
65. Challah M, Nadaud S, Philippe M, Battle T, Soubrier F, Corman B, and Michel JB. Circulating and cellular markers of endothelial dysfunction with aging in rats. *Am J Physiol* 273: H1941–H1948, 1997.
66. Chance B, Sies H, and Boveris A. Hydroperoxide metabolism in mammalian organs. *Physiol Rev* 59: 527–605, 1979.
67. Channon KM. Tetrahydrobiopterin: regulator of endothelial nitric oxide synthase in vascular disease. *Trends Cardiovasc Med* 14: 323–327, 2004.
68. Chen AF, Ren J, and Miao CY. Nitric oxide synthase gene therapy for cardiovascular disease. *Jpn J Pharmacol* 89: 327–336, 2002.
69. Chen XL, Varner SE, Rao AS, Grey JY, Thomas S, Cook CK, Wasserman MA, Medford RM, Jaiswal AK, and Kunsch C. Laminar flow induction of antioxidant response element-mediated genes in endothelial cells: a novel anti-inflammatory mechanism. *J Biol Chem* 278: 703–711, 2003.
70. Cheng C, Tempel D, van Haperen R, van der Baan A, Grosveld F, Daemen MJ, Krams R, and de Crom R. Atherosclerotic lesion size and vulnerability are determined by patterns of fluid shear stress. *Circulation* 113: 2744–2753, 2006.
71. Chung HY, Song SH, Kim HJ, Ikeno Y, and Yu BP. Modulation of renal xanthine oxidoreductase in aging: gene expression and reactive oxygen species generation. *J Nutr Health Aging* 3: 19–23, 1999.
72. Cody RJ, Haas GJ, Binkley PF, Capers Q, and Kelley R. Plasma endothelin correlates with the extent of pulmonary hypertension in patients with chronic congestive heart failure. *Circulation* 85: 504–509, 1992.
73. Cohen RA. The endothelium-derived hyperpolarizing factor puzzle: a mechanism without a mediator? *Circulation* 111: 724–727, 2005.
74. Collins RG, Velji R, Guevara NV, Hicks MJ, Chan L, and Beaudet AL. P-Selectin or intercellular adhesion molecule (ICAM)-1 deficiency substantially protects against atherosclerosis in apolipoprotein E-deficient mice. *J Exp Med* 191: 189–194, 2000.
75. Collins T and Cybulsky MI. NF-kappaB: pivotal mediator or innocent bystander in atherogenesis? *J Clin Invest* 107: 255–264, 2001.
76. Consentino F, Sill JC, and Katusië ZS. Role of superoxide anions in the mediation of endothelium-dependent contractions. *Hypertension* 23: 229–235, 1994.
77. Cooke JP, Singer AH, Tsao P, Zera P, Rowan RA, and Billingham ME. Antiatherogenic effects of L-arginine in the hypercholesterolemic rabbit. *J Clin Invest* 90: 1168–1172, 1992.
78. Cooper CJ, Landzberg MJ, Anderson TJ, Charbonneau F, Creager MA, Ganz P, and Selwyn AP. Role of nitric oxide in the local regulation of pulmonary vascular resistance in humans. *Circulation* 93: 266–2671, 1996.
79. Costa NJ, Dahm CC, Hurrell F, Taylor ER, and Murphy MP. The interactions of mitochondrial thiols with nitric oxide. *Antioxid Redox Signal* 5: 291–305, 2003.
80. Cracowski JL, Minson CT, Salvat-Melis M, and Halliwill JR. Methodological issues in the assessment of skin microvascular endothelial function in humans. *Trends Pharmacol Sci* 27: 503–508, 2006.
81. Creager MA, Gallagher SJ, Girerd XJ, Coleman SM, Dzau VJ, and Cooke JP. L-Arginine improves endothelium-dependent vasodilation in hypercholesterolemic humans. *J Clin Invest* 90: 1248–1253, 1992.
82. Crow J and Beckman J. The role of peroxynitrite in nitric oxide-mediated toxicity. In: Koprowski H, Maeda H, eds. *The role of nitric oxide in physiology and pathophysiology*. Berlin: Springer-Verlag, 1995, pp 57–73.
83. Csiszar A, Smith KE, Koller A, Kaley G, Edwards JG, and Ungvari Z. Regulation of bone morphogenetic protein-2 expression in endothelial cells: role of nuclear factor-kappaB activation by tumor necrosis factor-alpha, H_2O_2, and high intravascular pressure. *Circulation* 111: 2364–2372, 2005.
84. Csiszar A, Ungvari Z, Edwards JG, Kaminski P, Wolin MS, Koller A, and Kaley G. Aging-induced phenotypic changes and oxidative stress impair coronary arteriolar function. *Circ Res* 90: 1159–1166, 2002.
85. Cybulsky MI, Iiyama K, Li H, Zhu S, Chen M, Iiyama M, V D, Gutierrez-Ramos JC, Connelly PW, and Milstone DS. A major role for VCAM-1, but not ICAM-1, in early atherosclerosis. *J Clin Invest* 107: 1255–1262, 2001.
86. Daugherty A, Dunn JL, Rateri DL, and Heinecke JW. Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. *J Clin Invest* 94: 437–444, 1994.
87. Davies MJ, Gordon JL, Gearing AJ, Pigott R, Woolf N, Katz D, and Kyriakopoulos A. The expression of the adhesion molecules ICAM-1, VCAM-1, PECAM, and E-selectin in human atherosclerosis. *J Pathol* 171: 223–229, 1993.
88. Davignon J and Ganz P. Role of endothelial dysfunction in atherosclerosis. *Circulation* 109: 11127–11132, 2004.
89. Davis ME, Cai H, Drummond GR, and Harrison DG. Shear stress regulates endothelial nitric oxide synthase expression through c-Src by divergent signalling pathways. *Circ Res* 89: 1073–1080, 2001.
90. De Agostini AI, Watkins SC, Slayter HS, Youssoufian H, and Rosenberg RD. Localization of anticoagulantly active heparin sulphate proteoglycans in vascular endothelium: antithrombin binding on cultured endothelial cells and perfused rat aorta. *J Cell Biol* 111: 1293–1304, 1990.
91. Demiryurek AT, Karamsetty MR, McPhaden AR, Wadsworth RM, Kane KA, and MacLean MR. Accumulation of nitrotyrosine correlates with endothelial NO synthase in pulmonary resistance arteries during chronic hypoxia in the rat. *Pulmon Pharmacol Ther* 13: 157–165, 2000.
92. Devaraj S, Xu DY, and Jialal I. C-reactive protein increases plasminogen activator inhibitor-1 expression and activity in human aortic endothelial cells: implications for the metabolic syndrome and atherothrombosis. *Circulation* 107: 398–404, 2003.
93. Didion SP and Faraci FM. Ceramide-induced impairment of endothelial function is prevented by CuZn superoxide dismutase overexpression. *Arterioscler Thromb Vasc Biol* 25: 90–95, 2005.
94. Didion SP, Kinzenbaw DA, and Faraci FM. Critical role for CuZn-superoxide dismutase in preventing angiotensin II-

induced endothelial dysfunction. *Hypertension* 46: 1147–1153, 2005.
95. Donaldson K, Tran L, Jimenez LA, Duffin R, Newby DE, Mills N, MacNee W, and Stone V. Combustion-derived nanoparticles: a review of their toxicology following inhalation exposure. *Part Fibre Toxicol* 2: 10–24, 2005.
96. Dong ZM, Brown AA, and Wagner DD. Prominent role of P-selectin in the development of advanced atherosclerosis in ApoE-deficient mice. *Circulation* 101: 2290–2295, 2000.
97. Doshi SN, McDowell IF, Moat SJ, Lang D, Newcombe RG, Kredan MB, Lewis MJ, and Goodfellow J**.** Folate improves endothelial function in coronary artery disease: an effect mediated by reduction of intracellular superoxide? Arterioscler Thromb Vasc Biol 21: 1196–1202, 2001.
98. Du XL, Edelstein D, Rossetti L, Fantus IG, Goldberg H, Ziyadeh F, Wu J, and Brownlee M. Hyperglycaemia-induced mitochondrial superoxide overproduction activates the hexosamine pathways and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. *Proc Nat Acad Sci U S A* 97: 12222–12226, 2000.
99. Duda M, Konior A, Klemenska E, and Beresewicz A. Preconditioning protects endothelium by preventing ET-1-induced activation of NADPH oxidase and xanthine oxidase in post-ischemic heart. *J Mol Cell Cardiol* 42: 400–410, 2007.
100. Dupuis J, Tardif JC, Cernacek P, and Theroux P. Cholesterol reduction rapidly improves endothelial function after acute coronary syndromes: the RECIFE (Reduction of Cholesterol in Ischemia and Function of the Endothelium) trial. *Circulation* 99: 3227–3233, 1999.
101. Durand E, Scoazec A, Lafont A, Boddaert J, Al Hajzen A, Addad F, Mirshahi M, Desnos M, Tedgui A, and Mallat Z. In vivo induction of endothelial apoptosis leads to vessel thrombosis and endothelial denudation: a clue to the understanding of the mechanisms of thrombotic plaque erosion. *Circulation* 109: 2503–2506, 2004.
102. Durante W, Johnson FK, and Johnson RA. Arginase: a critical regulator of nitric oxide synthesis and vascular function. *Clin Exp Pharmacol Physiol* 34: 906–911, 2007.
103. Dzau VJ, Gnecchi M, Pachori AS, Morello F, and Melo LG. Therapeutic potential of endothelial progenitor cells in cardiovascular diseases. *Hypertension* 46: 7–18, 2005.
104. Echtay KS, Murphy MP, Smith RA, Talbot DA, and Brand MD. Superoxide activates mitochondrial uncoupling protein 2 from the matrix side: studies using targeted antioxidants. *J Biol Chem* 277: 47129–47135, 2002.
105. Eddahibi S, Raffestin B, Pham I, Launay JM, Aegerter P, Sitbon M, and Adnot S. Treatment with 5-HT potentiates development of pulmonary hypertension in chronically hypoxic rats. *Am J Physiol* 272: H1173–H1181, 1997.
106. Eddahibi S, Springall D, Mannan M, Carville C, Chabrier PE, Levame M, Raffestin B, Polak J, and Adnot S. Dilator effect of endothelins in pulmonary circulation: changes associated with chronic hypoxia. *Am J Physiol* 265: L571–L580, 1993.
107. Eikelboom JW, Lonn E, Genest J Jr, Hankey G, and Yusuf S. Homocyst(e)ine and cardiovascular disease: a critical review of the epidemiologic evidence. *Ann Intern Med* 131: 387–375, 1999.
108. Elmarakby AA, Loomis ED, Pollock JS, and Pollock DM. NADPH oxidase inhibition attenuates oxidative stress but not hypertension produced by chronic ET-1. *Hypertension* 45: 283–287, 2005.
109. Elmedal B, de Dam MY, Mulvany MJ, and Simonsen U. The superoxide dismutase mimetic, tempol, blunts right ventricular hypertrophy in chronic hypoxic rats. *Br J Pharmacol* 141: 105–113, 2004.
110. Enos WF, Holmes RH, and Beyer J. Coronary disease among United States soldiers killed in action in Korea: preliminary report. *JAMA* 152: 1090–1093, 1953.
111. Ergul A, Johansen JS, Strømhaug C, Harris AK, Hutchinson J, Tawfik A, Rahimi A, Rhim E, Wells B, Caldwell RW, and Anstadt MP. Vascular dysfunction of venous bypass conduits is mediated by reactive oxygen species in diabetes: role of endothelin-1. *J Pharmacol Exp Ther* 313: 70–77, 2005.
112. Erzen B, Gradisek P, Poredos P, and Sabovic M. Treatment of essential arterial hypertension with enalapril does not result in normalization of endothelial dysfunction of the conduit arteries. *Angiology* 57: 187–192, 2006.
113. Evans JL, Goldfine ID, Maddux BA, and Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr Rev* 23: 599–622, 2002.
114. Everson WV and Smart EJ. Influence of caveolin, cholesterol, and lipoproteins on nitric oxide synthase: implications for vascular disease. *Trends Cardiovasc Med* 11: 246–250, 2001.
115. Fagan KA, Morrissey B, Fouty BW, Sato K, Harral JW, Morris KG Jr, Hoedt-Miller M, Vidmar S, McMurtry IF, and Rodman DM. Upregulation of nitric oxide synthase in mice with severe hypoxia-induced pulmonary hypertension. *Respir Res* 2: 306–313, 2001.
116. Farb A, Burke AP, Tang AL, Liang TY, Mannan P, Smialek J, and Virmani R. Coronary plaque erosion without rupture into a lipid core: a frequent cause of coronary thrombosis in sudden coronary death. *Circulation* 93: 1354–1363, 1996.
117. Feigin VL, Anderson CS, and Mhurchu CN. Systemic inflammation, endothelial dysfunction, dietary fatty acids and micronutrients as risk factors for stroke: a selective review. *Cerebrovasc Dis* 13: 219–224, 2002.
118. Feletou M and Vanhoutte PM. Endothelium-derived hyperpolarizing factor: where are we now? *Arterioscler Thromb Vasc Biol* 26: 1215–1225, 2006.
119. Fennell JP, Brosnan MJ, Frater AJ, Hamilton CA, Alexander MY, Nicklin SA, Heistad DD, Baker AH, and Dominiczak AF. Adenovirus-mediated overexpression of extracellular superoxide dismutase improves endothelial dysfunction in a rat model of hypertension. *Gene Ther* 9: 110–117, 2002.
120. Fiege B, Ballhausen D, Kierat L, Leimbacher W, Goriounov D, Schircks B, Thony B, and Blau N. Plasma tetrahydrobiopterin and its pharmacokinetic following oral administration. *Mol Genet Metab* 81: 45–51, 2004.
121. Fisslthaler B, Hinsch N, Chataigneau T, Popp R, Kiss L, Busse R, and Fleming I. Nifedipine increases cytochrome P4502C expression and endothelium-derived hyperpolarizing factor-mediated responses in coronary arteries. *Hypertension* 36: 270–275, 2000.
122. Flammer AJ, Hermann F, Sudano I, Spieker L, Hermann M, Cooper KA, Serafini M, Luscher TF, Ruschitzka F, Noll G, and Corti R. Dark chocolate improves coronary vasomotion and reduces platelet reactivity. *Circulation* 116: 2376–2382, 2007.
123. Fleming I. Myoendothelial gap junctions: the gap is there, but does EDHF go through it? *Circ. Res* 86: 249–250, 2000.
124. Fleming I and Busse R. Endothelium-derived epoxyeicosatrienoic acids and vascular function. *Hypertension* 47: 629–633, 2006.
125. Flitney FW, Megson IL, Thomson JL, Kennovin GD, and Butler AR. Vasodilator responses of rat isolated tail artery enhanced by oxygen- dependent, photochemical release of

nitric oxide from iron-sulphur-nitrosyls. *Br J Pharmacol* 117: 1549–1557, 1996.
126. Flowers MA, Wang Y, Stewart RJ, Patel B, and Marsden PA. Reciprocal regulation of endothelin-1 and endothelial constitutive NOS in proliferating endothelial cells. *Am J Physiol* 269: H1988–H1997, 1995.
127. Forman MB, Puett DW, and Virmani R. Endothelial and myocardial injury during ischemia and reperfusion: pathogenesis and therapeutic implications. *J Am Coll Cardiol* 13: 2: 450–459, 1989.
128. Francis GS, Benedict C, Johnstone DE, Kirlin PC, Nicklas J, Liang CS, Kubo SH, Rudin-Toretsky E, and Yusuf S. Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart failure: a substudy of the Studies of Left Ventricular Dysfunction (SOLVD). *Circulation* 82: 1724–1729, 1990.
129. Frank PG, Woodman SE, Park DS, and Lisanti MP. Caveolin, caveolae, and endothelial cell function. *Arterioscler Thromb Vasc Biol* 23: 1161–1168, 2003.
130. Frankel EN. Browning and glycation reaction products in biology. In: Barnes PJ, and assoc. eds *Antioxidants in food and biology: facts and fiction.* Bridgwater, The Oily Press, 2007:193–216.
131. Franklin SS, Larson MG, Khan SA, Wong ND, Leip EP, Kannel WB, and Levy D. Does the relation of blood pressure to coronary heart disease risk change with aging? The Framingham Heart Study. *Circulation* 103: 1245–1249, 2001.
132. Furchgott RF and Jothianandan D. Endothelium-dependent and -independent vasodilation involving cyclic GMP: relaxation induced by nitric oxide, carbon monoxide and light. *Blood Vessels* 28: 52–61, 1991.
133. Furchgott RF and Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288: 373–376, 1980.
134. Garcin ED, Bruns CM, Lloyd SJ, Hosfield DJ, Tiso M, Gachhui R, Stuehr DJ, Tainer JA, and Getzoff ED. Structural basis for isozyme-specific regulation of electron transfer in nitric-oxide synthase. *J Biol Chem* 279: 37918–37927, 2004.
135. Gasser R, Koppel H, Brussee H, Grisold M, Holzmann S, and Klein W. EDRF does not mediate coronary vasodilation secondary to simulated ischemia: a study on KATP channels and N omega-nitro-L-arginine on coronary perfusion pressure in isolated Langendorff-perfused guinea-pig hearts. *Cardiovasc Drugs Ther* 12: 279–284, 1998.
136. Gauthier-Rein KM, Bizub DM, Lombard JH, and Rusch NJ. Hypoxia-induced hyperpolarization is not associated with vasodilation of bovine coronary resistance arteries. *Am J Physiol* 272: H1462–H1469, 1997.
137. Geiszt M, Kopp JB, Varnai P, and Leto TL. Identification of renox, an NAD(P)H oxidase in kidney. *Proc Natl Acad Sci U S A* 97: 8010–8014, 2000.
138. Genest JJ Jr, McNamara JR, Salem DN, Wilson PW, Schaefer EJ, and Malinow MR. Plasma homocyst(e)ine levels in men with premature coronary artery disease. *J Am Coll Cardiol* 16: 1114–1119, 1990.
139. Giaid A and Saleh D. Reduced expression of endothelial nitric oxide synthase in the lungs of patients with pulmonary hypertension. *N Engl J Med* 333: 214–221, 1995.
140. Giordano FJ. Oxygen, oxidative stress, hypoxia, and heart failure. *J Clin Invest* 115: 500–508, 2005.
141. Gladwin MT, Raat NJ, Shiva S, Dezfulian C, Hogg N, Kim-Shapiro DB, and Patel RP. Nitrite as a vascular endocrine nitric oxide reservoir that contributes to hypoxic signaling, cytoprotection, and vasodilation. *Am J Physiol Heart Circ Physiol* 291: H2026–H2035, 2006.
142. Gladwin MT, Schechter AN, Kim-Shapiro DB, Patel RP, Hogg N, Shiva S, Cannon RO 3rd, Kelm M, Wink DA, Espey MG, Oldfield EH, Pluta RM, Freeman BA, Lancaster JR Jr, Feelisch M, and Lundberg JO. The emerging biology of the nitrite anion. *Nat Chem Biol* 1: 308–314, 2005.
143. Goettsch W, Lattmann T, Amann K, Szibor M, Morawietz H, Munter K, Muller SP, Shaw S, and Barton M. Increased expression of endothelin-1 and inducible nitric oxide synthase isoform II in aging arteries in vivo: implications for atherosclerosis. Biochem Biophys Res Commun 280: 908–913, 2001.
144. Gojova A, Guo B, Kota RS, Rutledge JC, Kennedy IM, and Barakat AI. Induction of inflammation in vascular endothelial cells by metal oxide nanoparticles: effect of particle composition. *Environ Health Perspect* 115: 403–409, 2007.
145. Goldberg MS, Burnett RT, Valois MF, Flegel K, Bailar JC 3rd, Brook J, Vincent R, and Radon K. Associations between ambient air pollution and daily mortality among persons with congestive heart failure. *Environ Res* 91: 8–20, 2003.
146. Golino P, Piscione F, Benedict CR, Anderson HV, Cappelli-Bigazzi M, Indolfi C, Condorelli M, Chiariello M, and Willerson JT. Local effect of serotonin released during coronary angioplasty. *N Engl J Med* 330: 523–528, 1994.
147. Gorlach A, Brandes RP, Nguyen K, Amidi M, Deghani F, and Busse R. A gp91phox containing NADPH oxidase selectively expressed in endothelial cells is a major source of oxygen radical generation in the arterial wall. *Circ Res* 87: 26–32, 2000.
148. Gow AJ and Stamler JS. Reactions between nitric oxide and haemoglobin under physiological conditions. *Nature* 391: 169–173, 1998.
149. Goyal P, Weissmann N, Grimminger F, Hegel C, Bader L, Rose F, Fink L, Ghofrani HA, Schermuly RT, Schmidt HH, Seeger W, and Hanze J. Upregulation of NAD(P)H oxidase 1 in hypoxia activates hypoxia-inducible factor 1 via increase in reactive oxygen species. *Free Radic Biol Med* 36: 1279–1288, 2004.
150. Graser T and Vanhoutte PM. Hypoxic contraction of canine coronary arteries: role of endothelium and cGMP. *Am J Physiol* 261: H1769–H1777, 1991.
151. Gray DW and Marshall I. Novel signal transduction pathway mediating endothelium-dependent beta-adrenoceptor vasorelaxation in rat thoracic aorta. *Br J Pharmacol* 107: 684–690, 1992.
152. Gray GA and Webb DJ. The endothelin system and its potential as a therapeutic target in cardiovascular disease. *Pharmacol Ther* 72: 109–148, 1996.
153. Green K, Brand MD, and Murphy MP. Prevention of mitochondrial oxidative damage as a therapeutic strategy in diabetes. *Diabetes* 53(suppl 1): S110–S118, 2004.
154. Greenacre SA and Ischiropoulos H. Tyrosine nitration: localisation, quantification, consequences for protein function and signal transduction. *Free Radic Res* 34: 541–581, 2001.
155. Griendling KK, Minieri CA, Ollerenshaw JD, and Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res* 74: 1141–1148, 1994.
156. Griese DP, Ehsan A, Melo LG, Kong D, Zhang L, Mann MJ, Pratt RE, Mulligan RC, and Dzau VJ. Isolation and transplantation of autologous circulating endothelial cells into denuded vessels and prosthetic grafts: implications for cell-based vascular therapy. *Circulation* 108: 2710–2715, 2003.
157. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarcto miocardico. Dietary supplementation with n-3

polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Preventione trials. *Lancet* 354: 447–455, 1999.
158. Gryglewski RJ, Palmer RM, and Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. *Nature* 320: 454–456, 1986.
159. Guha M, Bai W, Nadler JL, and Natarajan R. Molecular mechanisms of tumor necrosis factor alpha gene expression in monocytic cells via hyperglycemia-induced oxidant stress-dependent and -independent pathways. *J Biol Chem* 275: 17728–17739, 2000.
160. Halcox JP, Nour KR, Zalos G, Mincemoyer RA, Waclawiw M, Rivera CE, Willie G, Ellahham S, and Quyyumi AA. The effect of sildenafil on human vascular function, platelet activation, and myocardial ischemia. *J Am Coll Cardiol* 40: 1232–1240, 2002.
161. Halcox JP, Schenke WH, Zalos G, Mincemoyer R, Prasad A, Waclawiw MA, Nour KR, and Quyyumi AA. Prognostic value of coronary vascular endothelial dysfunction. *Circulation* 106: 653–658, 2002.
162. Hansen CS, Sheykhzade M, Moller P, Folkmann JK, Amtorp O, Jonassen T and Loft S. Diesel exhaust particles induce endothelial dysfunction in apoE-/- mice. *Toxicol Appl Pharmacol* 219: 24–32, 2007.
163. Hanss M and Collen D. Secretion of tissue-type plasminogen activator and plasminogen activator inhibitor by cultured human endothelial cells: modulation by thrombin, endotoxin, and histamine. *J Lab Clin Med* 109: 97–104, 1987.
164. Harper JA, Dickinson K, and Brand MD. Mitochondrial uncoupling as a target for drug development for the treatment of obesity. *Obes Rev* 2: 255–265, 2001.
165. Harrison DG. Cellular and molecular mechanisms of endothelial cell dysfunction. *J Clin Invest* 100: 2153–2157, 1997.
166. Hassoun PM, Yu FS, Shedd AL, Zulueta JJ, V T, Lanzillo JJ, and Fanburg BL. Regulation of endothelial cell xanthine dehydrogenase xanthine oxidase gene expression by oxygen tension. *Am J. Physiol* 266: L163–L171, 1994.
167. Hatakeyama H, Miyamori I, Yamagishi S, Takeda Y, Takeda R, and Yamamoto H. Angiotensin II up-regulates the expression of type A endothelin receptor in human vascular smooth muscle cells. *Biochem Mol Biol Int* 34: 127–134, 1994.
168. Haynes WG, Hand MF, Johnstone HA, Padfield PL, and Webb DJ. Direct and sympathetically mediated venoconstriction in essential hypertension: enhanced responses to endothelin-1. *J Clin Invest* 94: 1359–1364, 1994.
169. He GW. Hyperkalemia exposure impairs EDHF-mediated endothelial function in the human coronary artery. *Ann Thorac Surg* 63: 84–87, 1997.
170. Heinecke JW. Oxidative stress: new approaches to diagnosis and prognosis in atherosclerosis. *Am J Cardiol* 91: 12A–16A, 2003.
171. Heiss C, Lauer T, Dejam A, Kleinbongard P, Hamada S, Rassaf T, Matern S, Feelisch M, and Kelm M. Plasma nitroso compounds are decreased in patients with endothelial dysfunction. *J Am Coll Cardiol* 47: 573–579, 2006.
172. 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.
173. 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.
174. Hennekens CH, Buring JE, Manson JE, Stampfer M, Rosner B, Cook NR, Belanger C, LaMotte F, Gaziano JM, Ridker PM, Willett W, and Peto R. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *N Engl J Med* 334: 1145–1149, 1996.
175. Herve P, Launay JM, Scrobohaci ML, Brenot F, Simonneau G, Petitpretz P, Poubeau P, Cerrina J, Duroux P, and Drouet L. Increased plasma serotonin in primary pulmonary hypertension. *Am J Med* 99: 249–254, 1995.
176. Higashi Y, Sasaki S, Nakagawa K, Matsuura H, Chayama K, and Oshima T. Effect of obesity on endothelium-dependent, nitric oxide-mediated vasodilation in normotensive individuals and patients with essential hypertension. *Am J Hypertens* 14: 1038–1045, 2001.
177. Higashi Y, Sasaki S, Nakagawa K, Matsuura H, Kajiyama G, and Oshima T. Effect of the angiotensin-converting enzyme inhibitor imidapril on reactive hyperemia in patients with essential hypertension: relationship between treatment periods and resistance artery endothelial function. *J Am Coll Cardiol* 37: 863–870, 2001.
178. Hilgers RH, Todd J Jr, and Webb RC. Regional heterogeneity in acetylcholine-induced relaxation in rat vascular bed: role of calcium-activated K+ channels. *Am J Physiol Heart Circ Physiol* 291: H216–H222, 2006.
179. Hogikyan RV, Galecki AT, Pitt B, Halter JB, Greene DA, and Supiano MA. Specific impairment of endothelium-dependent vasodilation in subjects with type 2 diabetes independent of obesity. *J Clin Endocrinol Metab* 83: 1946–1952, 1998.
180. Horiuchi S. Advanced glycation end products (AGE)-modified proteins and their potential relevance to atherosclerosis. *Trends Cardiovasc Med* 6: 163–168, 1996.
181. Hornig B and Drexler H. Endothelial function and bradykinin in humans. *Drugs* 54: 42–47, 1997.
182. Hoshikawa Y, Voelkel NF, Gesell TL, Moore MD, Morris KG, Alger LA, Narumiya S, and Geraci MW. Prostacyclin receptor-dependent modulation of pulmonary vascular remodeling. *Am J Respir Crit Care* 164: 314–318, 2001.
183. Hosoki R, Matsuki N, and Kimura H. the possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. *Biochem Biophys Res Commun* 237: 527–531, 1997.
184. Houstis N, Rosen ED, and Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature* 440: 944–948, 2006.
185. Hrafnkelsdottir T, Ottosson P, Gudnason T, Samuelsson O, and Jern S. Impaired endothelial release of tissue-type plasminogen activator in patients with chronic kidney disease and hypertension. *Hypertension* 44: 300–304, 2004.
186. Humbert M, Monti G, Brenot F, Sitbon O, Portier A, Grangeot-Keros L, Duroux P, Galanaud P, Simonneau G, and Emilie D. Increased interleukin-1 and interleukin-6 serum concentrations in severe primary pulmonary hypertension. *Am J Respir Crit Care Med* 151: 1628–1631, 1995.
187. Huo Y and Ley K. Adhesion molecules and atherogenesis. *Acta Physiol Scand* 173: 35–43, 2001.
188. Hussain AS, Marks GS, Brien JF, and Nakatsu K. The soluble guanylyl cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-alpha]quinoxalin-1-one (ODQ) inhibits relaxation of rabbit aortic rings induced by carbon monoxide, nitric oxide, and glyceryl trinitrate. *Can J Physiol Pharmacol* 75: 1034–1037, 1997.
189. Ichinose F, Roberts JD Jr, and Zapol WM. Inhaled nitric oxide: a selective pulmonary vasodilator: current uses and therapeutic potential. *Circulation* 109: 3106–3111, 2004.

190. Ihling C, Szombathy T, Bohrmann B, Brockhaus M, Schaefer HE and Loeffler BM. Coexpression of endothelin-converting enzyme-1 and endothelin-1 in different stages of human atherosclerosis. *Circulation* 104: 864–869, 2001.
191. Irodova NL, Lankin VZ, Konovalova GK, Kochetov AG, and Chazova IE. Oxidative stress in patients with primary pulmonary hypertension. *Bull Exp Biol Med* 133: 580–582, 2002.
192. Jaggar RT, Chan HY, Harris AL, and Bicknell R. Endothelial cell-specific expression of tumor necrosis factor-alpha from the KDR or E-selectin promoters following retroviral delivery. *Hum Gene Ther* 8: 2239–2247, 1997.
193. James AM and Murphy MP. How mitochondrial damage affects cell function. *J Biomed Sci* 9: 475–487, 2002.
194. Joannides R, Bizet-Nafeh C, Costentin A, Iacob M, Derumeaux G, Cribier A, and Thuillez C. Chronic ACE inhibition enhances the endothelial control of arterial mechanics and flow-dependent vasodilatation in heart failure. *Hypertension* 38: 1446–1450, 2001.
195. Joannides R, Haefeli WE, Linder L, Richard V, Bakkali EH, Thuillez C, and Luscher TF. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation* 91: 1314–1319, 1995.
196. Johnson RA and Johnson FK. Heme oxygenase-derived endogenous carbon monoxide impairs flow-induced dilation in resistance vessels. *Shock* 2007 (in press).
197. Jost S, Nolte CW, Sturm M, Hausleiter J, and Hausmann D. How to standardize vasomotor tone in serial studies based on quantitation of coronary dimensions? *Int J Cardiol Imaging* 14: 357–372, 1998.
198. Jourd'heuil D, Jourd'heuil FL, and Feelisch M. Oxidation and nitrosation of thiols at low micromolar exposure to nitric oxide: evidence for a free radical mechanism. *J Biol Chem* 278: 15720–15726, 2003.
199. Kaminski KA, Bonda TA, Korecki J, and Musial WJ. Oxidative stress and neutrophil activation: the two keystones of ischemia/reperfusion injury. *Int J Cardiol* 86: 41–59, 2002.
200. Kanse SM, Takahashi K, Lam HC, Rees A, Warren JB, Porta M, Molinatti P, Ghatei M, and Bloom SR. Cytokine stimulated endothelin release from endothelial cells. *Life Sci* 48: 1379–1384, 1991.
201. Karatzi K, Papamichael C, Karatzis E, Papaioannou TG, Voidonikola PT, Lekakis J, and Zampelas A. Acute smoking induces endothelial dysfunction in healthy smokers: is this reversible by red wine's antioxidant constituents? *J Am Coll Nutr* 26: 10–15, 2007.
202. Katusic ZS. Vascular endothelial dysfunction: does tetrahydrobiopterin play a role? *Am J Physiol Heart Circ Physiol* 281: H981–H986, 2001.
203. Katz SD, Balidemaj K, Homma S, Wu H, Wang J, and Maybaum S. Acute type 5 phosphodiesterase inhibition with sildenafil enhances flow-mediated vasodilation in patients with chronic heart failure. *J Am Coll Cardiol* 36: 845–851, 2000.
204. Katz SD, Rao R, Berman JW, Schwarz M, Demopoulos L, Bijou R, and LeJemtel TH. Pathophysiological correlates of increased serum tumor necrosis factor in patients with congestive heart failure: relation to nitric oxide-dependent vasodilation in the forearm circulation. *Circulation* 90: 12–16, 1994.
205. Kawaguchi H, Sawa H, and Yasuda H. Endothelin stimulates angiotensin I to angiotensin II conversion in cultured pulmonary artery endothelial cells. *J Mol Cell Cardiol* 22: 839–842, 1990.
206. Kawano H, Yasue H, Kitagawa A, Hirai N, Yoshida T, Soejima H, Miyamoto S, Nakano M, and Ogawa H. Dehydroepiandrosterone supplementation improves endothelial function and insulin sensitivity in men. *J Clin Endocrinol Metab* 88: 3190–3195, 2003.
207. Kawashima S and Yokoyama M. Dysfunction of endothelial nitric oxide synthase and atherosclerosis. *Arterioscler Thromb Vasc Biol* 24: 998–1005, 2004.
208. Keegan A, Morecroft I, Smillie D, Hicks MN, and MacLean MR. Contribution of the 5-HT(1B) receptor to hypoxia-induced pulmonary hypertension: converging evidence using 5-HT(1B)-receptor knockout mice and the 5-HT(1B/1D)-receptor antagonist GR127935. *Circ Res* 89: 1231–1239, 2001.
209. Kelly AS, Thelen AM, Kaiser DR, Gonzalez-Campoy JM, and Bank AJ. Rosiglitazone improves endothelial function and inflammation but not asymmetric dimethylarginine or oxidative stress in patients with type 2 diabetes mellitus. *Vasc Med* 12: 311–318, 2007.
210. Kelso GF, Porteous CM, Coulter CV, Hughes G, Porteous WK, Ledgerwood EC, Smith RAJ, and Murphy MP. Selective targeting of a redox-active ubiquinone to mitochondria within cells. *J Biol Chem* 276: 4588–4596, 2001.
211. Kerkhof CJ, Van Der Linden PJ, and Sipkema P. Role of myocardium and endothelium in coronary vascular smooth muscle responses to hypoxia. *Am J Physiol Heart Circ Physiol* 282: H1296–H1303, 2002.
212. Khechai F, Ollivier V, Bridey F, Amar M, Hakim J, and de Prost D. Effect of advanced glycation end product-modified albumin on tissue factor expression by monocytes: role of oxidant stress and protein tyrosine kinase activation. *Arterioscler Thromb Vasc Biol* 17: 2885–2890, 1997.
213. Khoo JP, Zhao L, Alp NJ, Bendall JK, Nicoli T, Rockett K, Wilkins MR, and Channon KM. Pivotal role for endothelial tetrahydrobiopterin in pulmonary hypertension. *Circulation* 111: 2126–2133, 2005.
214. Kim JA, Montagnani M, Koh KK, and Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. *Circulation* 113: 1888–1904, 2006.
215. Kimura M, Higashi Y, Hara K, Noma K, Sasaki S, Nakagawa K, Goto C, Oshima T, Yoshizumi M, and Chayama K. PDE5 inhibitor sildenafil citrate augments endothelium-dependent vasodilation in smokers. *Hypertension* 41: 1106–1110, 2003.
216. Kinlay S, Behrendt D, Wainstein M, Beltrame J, Fang JC, Creager MA, Selwyn AP and Ganz P. Role of endothelin-1 in the active constriction of human atherosclerotic coronary arteries. *Circulation* 104: 1114–1118, 2001.
217. Kiowski W, Sutsch G, Hunziker P, Muller P, Kim J, Oechslin E, Schmitt R, Jones R, and Bertel O. Evidence for endothelin-1-mediated vasoconstriction in severe chronic heart failure. *Lancet* 346: 732–736, 1995.
218. Kirstein M, Aston C, Hintz R, and Vlassara H. Receptor-specific induction of insulin-like growth factor I in human monocytes by advanced glycosylation end product-modified proteins. *J Clin Invest* 90: 439–446, 1992.
219. Klebanoff SJ. Oxygen metabolism and the toxic properties of phagocytes. *Ann Intern Med* 93: 480–489, 1980.
220. Klebanoff SJ. Reactive nitrogen intermediates and antimicrobial activity: role of nitrite. *Free Radic Biol Med* 14: 351–360, 1993.
221. Kleinbongard P, Dejam A, Lauer T, Jax T, Kerber S, Gharini P, Balzer J, Zotz RB, Scharf RE, Willers R, Schechter AN, Feelisch M, and Kelm M. Plasma nitrite concentrations reflect the degree of endothelial dysfunction in humans. *Free Radic Biol Med* 40: 295–302, 2006.

222. Kleinbongard P, Dejam A, Lauer T, Rassaf T, Schindler A, Picker O, Scheeren T, Godecke A, Schrader J, Schulz R, Heusch G, Schaub GA, Bryan NS, Feelisch M, and Kelm M. Plasma nitrite reflects constitutive nitric oxide synthase activity in mammals. *Free Radic Biol Med* 35: 790–796, 2003.
223. Kleinert H, Wallerath T, Euchenhofer C, Ihrig-Biedert I, Li H, and Fostermann U. Estrogens increase transcription of the human endothelial NO synthase gene: analysis of the transcription factors involved. *Hypertension* 31: 582–588, 1998.
224. Knekt P, Reunanen A, Jarvinen R, Seppanen R, Heliovaara M, and Aromaa A. Antioxidant vitamin intake and coronary mortality in a longitudinal population study. *Am J Epidemiol* 139: 1180–1189, 1994.
225. Knowles RG and Moncada S. Nitric oxide synthases in mammals. *Biochem J* 298: 249–258, 1994.
226. Kobayashi T, Tahara Y, Matsumoto M, Iguchi M, Sano H, Murayama T, Arai H, Oida H, Yurugi-Kobayashi T, Yamashita JK, Katagiri H, Majima M, Yokode M, Kita T, and Narumiya S. Roles of thromboxane A(2) and prostacyclin in the development of atherosclerosis in apoE-deficient mice. *J Clin Invest* 114: 784–794, 2004.
227. Konishi C, Naito Y, Saito Y, Ohara N, and Ono H. Age-related differences and roles of endothelial nitric oxide and prostanoids in angiotensin II responses of isolated, perfused mesenteric arteries and veins of rats. *Eur J Pharmacol* 320: 175–181, 1997.
228. Kukovetz WR, Holzmann S, Wurm A, and Poch G. Prostacyclin increases cAMP in coronary arteries. *J Cyclic Nucleotide Res* 5: 469–476, 1979.
229. Kushi LH, Folsom AR, Prineas RJ, Mink PJ, Wu Y, and Bostick RM. Dietary antioxidant vitamins and death from coronary heart disease in post-menopausal women. *N Engl J Med* 334: 1156–1162, 1996.
230. Lacza Z, Puskar M, Kis B, Perciaccante JV, Miller AW, and Busija DW. Hydrogen peroxide acts as an EDHF in the piglet pial vasculature in response to bradykinin. *Am J Physiol Heart Circ Physiol* 283: H406–H411, 2002.
231. Lander HM, Tauras JM, Ogiste JS, Hori O, Moss RA, and Schmidt AM. Activation of the receptor for advanced glycation end products triggers a p21(ras)-dependent mitogen-activated protein kinase pathway regulated by oxidant stress. *J Biol Chem* 272: 17810–17814, 1997.
232. Landmesser U, Bahlmann F, Mueller M, Spiekermann S, Kirchhoff N, Schulz S, Manes C, Fischer D, de Groot K, Fliser D, Fauler G, Marz W, and Drexler H. Simvastatin *versus* ezetimibe: pleiotropic and lipid-lowering effects on endothelial function in humans. *Circulation* 111: 2356–2363, 2005.
233. Landmesser U, Cai H, Dikalov S, McCann L, Hwang J, Jo H, Holland SM, and Harrison DG. Role of p47(phox) in vascular oxidative stress and hypertension caused by angiotensin II. *Hypertension* 40: 511–515, 2002.
234. Landmesser U, Merten R, Spiekermann S, Buttner K, Drexler H, and Hornig B. Vascular extracellular superoxide dismutase activity in patients with coronary artery disease: relation to endothelium-dependent vasodilation. *Circulation* 101: 2264–2270, 2000.
235. Lapu-Bula R and Ofili E. From hypertension to heart failure: role of nitric oxide-mediated endothelial dysfunction and emerging insights from myocardial contrast echocardiography. *Am J Cardiol* 99: 7D–14D, 2007.
236. Laukkanen MO, Kivela A, Rissanen T, Rutanen J, Karkkainen MK, Leppanen O, Brasen JH and Yla-Herttuala S. Adenovirus-mediated extracellular superoxide dismutase gene therapy reduces neointima formation in balloon-denuded rabbit aorta. *Circulation* 106: 1999–2003, 2002.
237. Laursen JB, Rajagopalan S, Galis Z, Tarpey M, Freeman BA, and Harrison DG. Role of superoxide in angiotensin II-induced but not catecholamine-induced hypertension. *Circulation* 95: 588–593, 1997.
238. Leffler CW, Parfenova H, Jaggar JH, and Wang R. Carbon monoxide and hydrogen sulphide: gaseous messengers in cerebrovascular circulation. *J Appl Physiol* 100: 1065–1076, 2006.
239. Leiper J and Vallance P. Biological significance of endogenous methylarginines that inhibit nitric oxide synthases. *Cardiovasc Res* 43: 542–548, 1999.
240. Lembo G, Iaccarino G, Vecchione C, Barbato E, Izzo R, Fontana D, and Trimarco B. Insulin modulation of an endothelial nitric oxide component present in the alpha2- and beta-adrenergic responses in human forearm. *J Clin Invest* 100: 2007–2014, 1997.
241. Lembo G, Iaccarino G, Vecchione C, Barbato E, Morisco C, Monti F, Parrella L, and Trimarco B. Insulin enhances endothelial alpha2-adrenergic vasorelaxation by a pertussis toxin mechanism. *Hypertension* 30: 1128–1134, 1997.
242. Lerman A, Suwaidi JA, and Velianou JL. L-Arginine: a novel therapy for coronary artery disease? *Expert Opin Invest Drugs* 8: 1785–1793, 1999.
243. Leslie SJ, Affolter J, Denvir MA, and Webb DJ. Validation of laser Doppler flowmetry coupled with intra-dermal injection for investigating effects of vasoactive agents on the skin microcirculation in man. *Eur J Clin Pharmacol* 59: 99–102, 2003.
244. Leslie SJ, Attina T, Hultsch E, Bolscher L, Grossman M, Denvir MA, and Webb DJ. Comparison of two plethysmography systems in assessment of forearm blood flow. *J Appl Physiol* 96: 1794–1799, 2004.
245. Leu HB, Charng MJ, and Ding PY. A double blind randomized trial to compare the effects of eprosartan and enalapril on blood pressure, platelets, and endothelium function in patients with essential hypertension. *Jpn Heart J* 45: 623–635, 2004.
246. Levin EG and Santell L. Stimulation and desensitization of tissue plasminogen activator release from human endothelial cells. *J Biol Chem* 263: 9360–9365, 1988.
247. Lewis RS and Deen WM. Kinetics of the reaction of nitric oxide with oxygen in aqueous solutions. *Chem Res Toxicol* 7: 568–574, 1994.
248. Li Volti G, Wang J, Traganos F, Kappas A, and Abraham NG. Differential effect of heme oxygenase-1 in endothelial and smooth muscle cell cycle progression. *Biochem Biophys Res Commun* 296: 1077–1082, 2002.
249. Li H, Chen SJ, Chen YF, Meng QC, Durand J, Oparil S, and Elton TS. Enhanced endothelin-1 and endothelin receptor gene expression in chronic hypoxia. *J Appl Physiol* 77: 1451–1459, 1994.
250. Li L, Crockett E, Wang DH, Galligan JJ, Fink GD, and Chen AF. Gene transfer of endothelial NO synthase and manganese superoxide dismutase on arterial vascular cell adhesion molecule-1 expression and superoxide production in deoxycorticosterone acetate-salt hypertension. *Arterioscler Thromb Vasc Biol* 22: 249–255, 2002.
251. Li L, Fink GD, Watts SW, Northcott CA, Galligan JJ, Pagano PJ, and Chen AF. Endothelin-1 increases vascular superoxide via endothelin(A)-NADPH oxidase pathway in low-renin hypertension. *Circulation* 107: 1053–1058, 2003.

252. Li LJ, Geng SR, and Yu CM. Endothelial dysfunction in normotensive Chinese with a family history of essential hypertension. *Clin Exp Hypertens* 27: 1–8, 2005.
253. Li N and Karin M. Is NF-kappaB the sensor of oxidative stress? *FASEB J* 13: 1137–1143, 1999.
254. Li YM, Tan AX, and Vlassara H. Antibacterial activity of lysozyme and lactoferrin is inhibited by binding of advanced glycation-modified proteins to a conserved motif. *Nat Med* 1: 1057–1061, 1995.
255. Liaudet L, Szabo G, and Szabo C. Oxidative stress and regional ischemia-reperfusion injury: the peroxynitrite-poly(ADP-ribose) polymerase connection. *Coron Artery Dis* 14: 115–122, 2003.
256. Libby P. Inflammation in atherosclerosis. *Nature* 420: 868–874, 2002.
257. Liem A, Reynierse-Buitenwerf GH, Zwinderman AH, Jukema JW, and van Veldhuisen DJ. Secondary prevention with folic acid: effects on clinical outcomes. *J Am Coll Cardiol* 41: 2105–2113, 2003.
258. Liu JQ and Folz RJ. Extracellular superoxide enhances 5-HT-induced murine pulmonary artery vasoconstriction. *Am J Physiol* 287: L111–L118, 2004.
259. Liu JQ, Zelko IN, Erbynn EM, Sham JS, and Folz RJ. Hypoxic pulmonary hypertension: role of superoxide and NADPH oxidase (gp91phox). *Am J Physiol* 290: L2–L10, 2006.
260. Liu Y, Terata K, Chai Q, Li H, Kleinman LH, and Gutterman DD. Peroxynitrite inhibits Ca2+-activated K+ channel activity in smooth muscle of human coronary arterioles. *Circ Res* 91: 1070–1076, 2002.
261. Lizasoain I, Moro MA, Knowles RG, Darley-Usmar V, and Moncada S. Nitric oxide and peroxynitrite exert distinct effects on mitochondrial respiration which are differentially blocked by glutathione or glucose. *Biochem J* 314: 877–880, 1996.
262. Lopez Farre A and Casado S. Heart failure, redox alterations, and endothelial dysfunction. *Hypertension* 38: 1400–1405, 2001.
263. Lucchesi BR. Complement activation, neutrophils, and oxygen radicals in reperfusion injury. *Stroke* 24: I41–I47,1993.
264. Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW and Ganz P. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med* 315: 1046–1051, 1986.
265. Lüscher TF, Boulanger CM, Dohu Y, and Yang ZH. Endothelium-derived contracting factors. *Hypertension* 19: 117–130, 1992.
266. Maguire JJ, Wilson DS, and Packer L. Mitochondrial electron transport-linked tocoperoxyl radical reduction. *J Biol Chem* 264: 21462–21465, 1989.
267. Maines MD. Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. *FASEB J* 2: 2557–2568, 1988.
268. Mancini GB, Henry GC, Macaya C, O'Neill BJ, Pucillo AL, Carere RG, Wargovich TJ, Mudra H, Luscher TF, Klibaner MI, Haber HE, Uprichard AC, Pepine CJ, and Pitt B. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease: the TREND (Trial on Reversing ENdothelial Dysfunction) Study. *Circulation* 94: 258–265, 1996.
269. Marcus AJ, Broekman MJ, Drosopoulos JHF, Islam N, Alyonycheva TN, Safier LB, Hajjar KA, Posnett DN, Schoenborn MA, Schooley KA, Gayle RB, and Maliszewski CR. The endothelial cell ecto-ADPase responsible for inhibition of platelet function is CD39. *J Clin Invest* 99: 1351–1360, 1997.
270. Markewitz BA, Michael JR, and Kohan DE. Endothelin-1 inhibits the expression of inducible nitric oxide synthase. *Am J Physiol* 272: L1078–L1083, 1997.
271. Mason RP, Walter MF, and Jacob RF. Effects of HMG-CoA reductase inhibitors on endothelial function: role of microdomains and oxidative stress. *Circulation* 109: II34–II41, 2004.
272. Mather KJ, Lteif A, Steinberg HO, and Baron AD. Interactions between endothelin and nitric oxide in the regulation of vascular tone in obesity and diabetes. *Diabetes* 53: 2060–2066, 2004.
273. Mather KJ, Mirzamohammadi B, Lteif A, Steinberg HO, and Baron AD. Endothelin contributes to basal vascular tone and endothelial dysfunction in human obesity and type 2 diabetes. *Diabetes* 51: 3517–3523, 2002.
274. Matoba T and Shimokawa H. Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in animals and humans. *J Pharmacol Sci* 92: 1–6, 2003.
275. Matoba T, Shimokawa H, Morikawa K, Kubota H, Kunihiro I, Urakami-Harasawa L, Mukai Y, Hirakawa Y, Akaike T, and Takeshita A. Electron spin resonance detection of hydrogen peroxide as an endothelium-derived hyperpolarizing factor in porcine coronary microvessels. *Arterioscler Thromb Vasc Biol* 23: 1224–1230, 2003.
276. Mavria G, Jager U, and Porter CD. Generation of a high titre retroviral vector for endothelial cell-specific gene expression in vivo. *Gene Ther* 7: 368–376, 2000.
277. McCance DR, Dyer DG, Dunn JA, Bailie KE, Thorpe SR, Baynes JW, and Lyons TJ. Maillard reaction products and their relation to complications in insulin-dependent diabetes mellitus. *J Clin Invest* 91: 2470–2478, 1993.
278. McCulloch KM, Docherty C, and MacLean MR. Endothelin receptors mediating contraction of rat and human pulmonary resistance arteries: effect of chronic hypoxia in the rat. *Br J Pharmacol* 123: 1621–1630, 1998.
279. McGuire JJ, Ding H, and Triggle CR. Endothelium-derived relaxing factors: a focus on endothelium-derived hyperpolarizing factor(s). *Can J Physiol Pharmacol* 79: 443–470, 2001.
280. McIntyre CA, Buckley CH, Jones GC, Sandeep TC, Andrews RC, Elliott AI, Gray GA, Williams BC, McKnight JA, Walker BR, and Hadoke PW. Endothelium-derived hyperpolarizing factor and potassium use different mechanisms to induce relaxation of human subcutaneous resistance arteries. *Br J Pharmacol* 133: 902–908, 2001.
281. McVeigh GE, Brennan GM, Johnston GD, McDermott BJ, McGrath LT, Henry WR, Andrews JW, and Hayes JR. Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 35: 771–776, 1992.
282. Megson IL, Holmes SA, Magid KS, Pritchard RJ, and Flitney FW. Selective modifiers of glutathione biosynthesis and 'repriming' of vascular smooth muscle photorelaxation. *Br J Pharmacol* 130: 1575–1580, 2000.
283. Megson IL and Webb DJ. Nitric oxide donor drugs: current status and future trends. *Expert Opin Invest Drugs* 11: 587–601, 2002.
284. Mehta J, Lopez L, Chen L, and Cox O. Alterations in nitric oxide activity, superoxide anion generation and platelet aggregation in systemic hypertension, and effect of celiprolol. *Am J Cardiol* 74: 901–905, 1994.
285. Melo LG, Gnecchi M, Pachori AS, Kong D, Wang K, Liu X, Pratt RE, and Dzau VJ. Endothelium-targeted gene and cell-

based therapies for cardiovascular disease. *Arterioscler Thromb Vasc Biol* 24: 1761–1774, 2004.
286. Michelakis ED, Tymchak W, Noga M, Webster L, Wu XC, Lien D, Wang SH, Modry D, and Archer SL. Long-term treatment with oral sildenafil is safe and improves functional capacity and hemodynamics in patients with pulmonary arterial hypertension. *Circulation* 108: 2066–2069, 2003.
287. Michiels C, Arnould T, Knott I, Dieu M, and Remacle J. Stimulation of prostaglandin synthesis by human endothelial cells exposed to hypoxia. *Am J Physiol* 264: C866–C874, 1993.
288. Miller AA, Megson IL, and Gray GA. Inducible nitric oxide synthase-derived superoxide contributes to hyperreactivity in small mesenteric arteries from a rat model of chronic heart failure. *Br J Pharmacol* 131: 29–36, 2000.
289. Miller MR and Megson IL. Recent developments in nitric oxide donor drugs. *Br J Pharmacol* 151: 305–321, 2007.
290. Mills NL, Amin N, Robinson SD, Anand A, Davies J, Patel D, de la Fuente JM, Cassee FR, Boon NA, Macnee W, Millar AM, Donaldson K, and Newby DE. Do inhaled carbon nanoparticles translocate directly into the circulation in humans? *Am J Respir Crit Care Med* 173: 426–431, 2006.
291. Mills NL, Tornqvist H, Robinson SD, Gonzalez M, Darnley K, MacNee W, Boon NA, Donaldson K, Blomberg A, Sandstrom T, and Newby DE. Diesel exhaust inhalation causes vascular dysfunction and impaired endogenous fibrinolysis. *Circulation* 112: 3930–3936, 2005.
292. Miyata T, Hori O, Zhang J, Yan SD, Ferran L, Iida Y, and Schmidt AM. The receptor for advanced glycation end products (RAGE) is a central mediator of the interaction of AGE-beta2microglobulin with human mononuclear phagocytes via an oxidant-sensitive pathway. Implications for the pathogenesis of dialysis-related amyloidosis. *J Clin Invest* 98: 1088–1094, 1996.
293. Modena MG, Bonetti L, Coppi F, Bursi F, and Rossi R. Prognostic role of reversible endothelial dysfunction in hypertensive postmenopausal women. *J Am Coll Cardiol* 40: 505–510, 2002.
294. Moens AL and Kass DA. Tetrahydrobiopterin and cardiovascular disease. *Arterioscler Thromb Vasc Biol* 26: 2439–2444, 2006.
295. Moncada S, Palmer RM, and Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 43: 109–142, 1991.
296. Morishita R, Sugimoto T, Aoki M, Kida I, Tomita N, Moriguchi A, Maeda K, Sawa Y, Kaneda Y, Higaki J, and Ogihara T. In vivo transfection of cis element "decoy" against nuclear factor-kappaB binding site prevents myocardial infarction. *Nat Med* 3: 894–899, 1997.
297. Morse JH. Bone morphogenetic protein receptor 2 mutations in pulmonary hypertension. *Chest* 121: 50S–53S, 2002.
298. Motomiya Y, Oyama N, Iwamoto H, Uchimura T, and Maruyama I. N epsilon-(carboxymethyl)lysine in blood from maintenance hemodialysis patients may contribute to dialysis-related amyloidosis. *Kidney Int* 54: 1357–1366, 1998.
299. Mukai Y, Shimokawa H, Higashi M, Morikawa K, Matoba T, Hiroki J, Kunihiro I, Talukder HMA, and Takeshita A. Inhibition of rennin-angiotensin system ameliorates endothelial dysfunction associated with aging in rats. *Arterioscler Thromb Vasc Biol* 22: 1445–1450, 2002.
300. Murad F. Shattuck Lecture: nitric oxide and cyclic GMP in cell signaling and drug development. *N Engl J Med* 355: 2003–2011, 2006.
301. Musameh MD, Fuller BJ, Mann BE, Green CJ, and Motterlini R. Positive inotropic effects of carbon monoxide-releasing molecules (CO-ROMs) in the isolated perfused rat heart. *Br J Pharmacol* 149: 1104–1112, 2006.
302. Muscat S, Pelka J, Hegele J, Weigle B, Munch G, and Pischetsrieder M. Coffee and Maillard products activate NF-kappaB in macrophages via H2O2 production. *Mol Nutr Food Res* 51: 525–535, 2007.
303. Nadar S, Blann AD, and Lip GY. Antihypertensive therapy and endothelial function. *Curr Pharm Des* 10: 3607–3614, 2004.
304. Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, Badimon JJ, Stefanadis C, Moreno P, Pasterkamp G, Fayad Z, Stone PH, Waxman S, Raggi P, Madjid M, Zarrabi A, Burke A, Yuan C, Fitzgerald PJ, Siscovick DS, de Korte CL, Aikawa M, Juhani Airaksinen KE, Assmann G, Becker CR, Chesebro JH, Farb A, Galis ZS, Jackson C, Jang IK, Koenig W, Lodder RA, March K, Demirovic J, Navab M, Priori SG, Rekhter MD, Bahr R, Grundy SM, Mehran R, Colombo A, Boerwinkle E, Ballantyne C, Insull W Jr, Schwartz RS, Vogel R, Serruys PW, Hansson GK, Faxon DP, Kaul S, Drexler H, Greenland P, Muller JE, Virmani R, Ridker PM, Zipes DP, Shah PK, and Willerson JT. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: part I. *Circulation* 108: 1664–1672, 2003.
305. Naik JS, O'Donaughy TL, and Walker BR. Endogenous carbon monoxide is an endothelial-derived vasodilator factor in the mesenteric circulation. *Am J Physiol Heart Circ Physiol* 284: H838–H845, 2003.
306. Nakao A, Choi AM, and Murase N. Protective effect of carbon monoxide in transplantation. *J Cell Mol Med* 10: 650–671, 2006.
307. Nakhostine N and Lamontagne D. Adenosine contributes to hypoxia-induced vasodilation through ATP-sensitive K+ channel activation. *Am J Physiol* 265: H1289–H1293, 1993.
308. Napoli C, de Nigris F, and Palinski W. Multiple role of reactive oxygen species in the arterial wall. *J Cell Biochem* 82: 674–682, 2001.
309. Neglia D, Michelassi C, Trivieri MG, Sambuceti G, Giorgetti A, Pratali L, Gallopin M, Salvadori P, Sorace O, Carpeggiani C, Poddighe R, L'Abbate A, and Parodi O. Prognostic role of myocardial blood flow impairment in idiopathic left ventricular dysfunction. *Circulation* 105: 186–193, 2002.
310. Ness A and Smith GD. Mortality in the CHAOS trial: Cambridge Heart Antioxidant Study. *Lancet* 353: 1017–1018, 1999.
311. Ness AR and Powles JW. Fruit and vegetables, and cardiovascular disease: a review. *Int J Epidemiol* 26: 1–13, 1997.
312. Neunteufl T, Heher S, Katzenschlager R, Wolfl G, Kostner K, Maurer G, and Weidinger F. Late prognostic value of flow-mediated dilation in the brachial artery of patients with chest pain. *Am J Cardiol* 86: 207–210, 2000.
313. Newby DE, McLeod AL, Uren NG, Flint L, Ludlam CA, Webb DJ, Fox KA, and Boon NA. Impaired coronary tissue plasminogen activator release is associated with coronary atherosclerosis and cigarette smoking: direct link between endothelial dysfunction and atherothrombosis. *Circulation* 103: 1936–1941, 2001.
314. Newby DE, Witherow FN, Wright RA, Bloomfield P, Ludlam CA, Boon NA, Fox KA, and Webb DJ. Hypercholesterolaemia and lipid lowering treatment do not affect the acute endogenous fibrinolytic capacity in vivo. *Heart* 87: 48–53, 2002.
315. Nicholls SJ and Hazen SJ. Myeloperoxidase and cardiovascular disease. *Arterioscler Thromb Vasc Biol* 25: 1102–1111, 2005.

316. Nicklin SA, Reynolds PN, Brosnan MJ, White SJ, Curiel DT, Dominiczak AF, and Baker AH. Analysis of cell-specific promoters for viral gene therapy targeted at the vascular endothelium. *Hypertension* 38: 65–70, 2001.
317. Niederhoffer N and Szabo B. Involvement of CB1 cannabinoid receptors in the EDHF-dependent vasorelaxation in rabbits. *Br J Pharmacol* 126: 1383–1386, 1999.
318. Nielsen WB, Vestbo J, and Jensen GB. Isolated systolic hypertension as a major risk factor for stroke and myocardial infarction and an unexploited source of cardiovascular prevention: a prospective population-based study. *J Hum Hypertens* 9: 175–180, 1995.
319. Nishikawa T, Edelstein D, Du XL, Yamagishi SI, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino A, and Brownlee M. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 404: 787–790, 2000.
320. Nissen SE and Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med* 356: 2457–2471, 2007.
321. Nohl H, Kozlov AV, Gille L, and Staniek K. Cell respiration and formation of reactive oxygen species: facts and artefacts. *Biochem Soc Trans* 31: 1308–1311, 2003.
322. Ohara Y, Peterson TE, and Harrison DG. Hypercholesterolemia increases endothelial superoxide anion production. *J Clin Invest* 91: 2546–2551, 1993.
323. Ohgami N, Nagai R, Ikemoto M, Arai H, Kuniyasu A, Horiuchi S, and Nakayama H. Cd36, a member of the class b scavenger receptor family, as a receptor for advanced glycation end products. *J Biol Chem* 276: 3195–3202, 2001.
324. Oliver JJ and Webb DJ. Noninvasive assessment of arterial stiffness and risk of atherosclerotic events. *Arterioscler Thromb Vasc Biol* 23: 554–566, 2003.
325. Oliver JJ, Webb DJ, and Newby DE. Stimulated tissue plasminogen activator release as a marker of endothelial function in humans. *Arterioscler Thromb Vasc Biol* 25: 2470–2479, 2005.
326. Opar A. Where now for new drugs for atherosclerosis? *Nat Rev Drug Discov* 6: 334–335, 2007.
327. Ortiz MC, Sanabria E, Manriquez MC, Romero JC, and Juncos LA. Role of endothelin and isoprostanes in slow pressor responses to angiotensin II. *Hypertension* 37: 505–510, 2001.
328. Ortwerth BJ, James H, Simpson G, and Linetsky M. The generation of superoxide anions in glycation reactions with sugars, osones and 3-deoxyosones. *Biochem Biophys Res Commun* 245: 1: 161–165, 1998.
329. Ozaki M, Kawashima S, Yamashita T, Ohashi Y, Rikitake Y, Inoue N, Hirata KI, Hayashi Y, Itoh H, and Yokoyama M. Reduced hypoxic pulmonary vascular remodeling by nitric oxide from the endothelium. *Hypertension* 37: 322–327, 2001.
330. Padro T, Emeis JJ, Steins M, Schmid KW, and Kienast J. Quantification of plasminogen activators and their inhibitors in the aortic vessel wall in relation to the presence and severity of atherosclerotic disease. *Arterioscler Thromb Vasc Biol* 15: 893–902, 1995.
331. Parker C 3rd, Vita JA, and Freedman JE. Soluble adhesion molecules and unstable coronary artery disease. *Atherosclerosis* 156: 417–424, 2001.
332. Patel S and Celermajer DS. Assessment of vascular disease using arterial flow mediated dilatation. *Pharmacol Rep* 58(suppl): 3–7, 2006.
333. 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.
334. Picchi A, Gao X, Belmadani S, Potter BJ, Focardi M, Chilian WM, and Zhang C. Tumor necrosis factor-alpha induces endothelial dysfunction in the prediabetic metabolic syndrome. *Circ Res* 99: 69–77, 2006.
335. Podrez EA, Abu-Soud HM, and Hazen SL. Myeloperoxidase-generated oxidants and atherosclerosis. *Free Radic Biol Med* 28: 1717–1725, 2000.
336. Pohl U and Busse R. Hypoxia stimulates release of endothelium-derived relaxant factor. *Am J Physiol* 256: H1595–H1600, 1989.
337. Pollock DM. Endothelin, angiotensin, and oxidative stress in hypertension. *Hypertension* 45: 477–480, 2005.
338. Pomposiello S, Rhaleb NE, Alva M, and Carretero OA. Reactive oxygen species: role in the relaxation induced by bradykinin or arachidonic acid via EDHF in isolated porcine coronary arteries. *J Cardiovasc Pharmacol* 34: 567–574, 1999.
339. Pyke KE and Tschakovsky ME. The relationship between shear stress and flow-mediated dilatation: implications for the assessment of endothelial function. *J Physiol* 568: 357–369, 2005.
340. Quillen JE, Sellke FW, Brooks LA, and Harrison DG. Ischemia-reperfusion impairs endothelium-dependent relaxation of coronary microvessels but does not affect large arteries. *Circulation* 82: 586–594, 1990.
341. Radomski MW, Palmer RM, and Moncada S. An L-arginine/nitric oxide pathway present in human platelets regulates aggregation. *Proc Natl Acad Sci U S A* 87: 5193–5197, 1990.
342. Raha H and Robinson BH. Mitochondria, oxygen free radicals, disease and ageing. *Trends Biochem* 25: 502–508, 2000.
343. 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.
344. Ranjan Z, Xiao Z, and Diamond SL. Constitutive NOS expression in cultured endothelial cells is elevated by fluid shear stress. *Am J Physiol* 269: H550–H555, 1995.
345. Rich S and McLaughlin VV. Endothelin receptor blockers in cardiovascular disease. *Circulation* 108: 2184–2190, 2003.
346. Richardson TB, Kaspers J, and Porter CD. Retroviral hybrid LTR vector strategy: functional analysis of LTR elements and generation of endothelial cell specificity. *Gene Ther* 11: 775–783, 2004.
347. Ridker PM. C-reactive protein and the prediction of cardiovascular events among those at intermediate risk: moving an inflammatory hypothesis toward consensus. *J Am Coll Cardiol* 49: 2129–2138, 2007.
348. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation* 107: 363–369, 2003.
349. Ridker PM, Rifai N, Rose L, Buring JE, and Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med* 347: 1557–1565, 2002.
350. Riethmuller C, Gorren AC, Pitters E, Hemmens B, Habisch HJ, Heales SJ, Schmidt K, Werner ER, and Mayer B. Activation of neuronal nitric-oxide synthase by the 5-methyl analog of tetrahydrobiopterin: functional evidence against reductive oxygen activation by the pterin cofactor. *J Biol Chem* 274: 16047–16051, 1999.

351. Rikitake Y and Liao JK. Rho GTPases, statins, and nitric oxide. *Circ Res* 97: 1232–1235, 2005.
352. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, and Willett WC. Vitamin E consumption and the risk of coronary heart disease in men. *N Engl J Med* 328: 1450–1456, 1993.
353. Riobo NA, Clementi E, Melani M, Boveris A, Cadenas E, Moncada S, and Poderoso JJ. Nitric oxide inhibits mitochondrial NADH:ubiquinone reductase activity through peroxynitrite formation. *Biochem J* 359: 139–145, 2001.
354. Robinson SD, Ludlam CA, Boon NA, and Newby DE. Phosphodiesterase type 5 inhibition does not reverse endothelial dysfunction in patients with coronary heart disease. *Heart* 92: 170–176, 2006.
355. Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med* 340: 115–126, 1999.
356. 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.
357. Rubanyi GM and Vanhoutte PM. Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. *Am J Physiol* 250: H822–H827, 1986.
358. Rubenfire M, Cao N, Smith DE, and Mosca L. Carotid artery reactivity to isometric hand grip exercise identifies persons at risk and with coronary disease. *Atherosclerosis* 160: 241–248, 2002.
359. Sakai K, Matsumoto K, Nishikawa T, Suefuji M, Nakamaru K, Hirashima Y, Kawashima J, Shirotani T, Ichinose K, Brownlee M, and Araki E. Mitochondrial reactive oxygen species reduce insulin secretion by pancreatic beta-cells. *Biochem Biophys Res Commun* 300: 216–222, 2003.
360. Salame MY, Samani NJ, Masood I, and deBono DP. Expression of the plasminogen activator system in the human vascular wall. *Atherosclerosis* 152: 19–28, 2000.
361. Salvemini D and Marino MH. Inducible nitric oxide synthase and inflammation. *Expert Opin Invest Drugs* 7: 65–75, 1998.
362. Sandow SL and Tare M. C-type natriuretic peptide: a new endothelium-derived hyperpolarizing factor? *Trends Pharmacol Sci* 28: 61–67, 2007.
363. Sandow SL, Tare M, Coleman HA, Hill CE, and Parkington HC. Involvement of myoendothelial gap junctions in the actions of endothelium-derived hyperpolarizing factor. *Circ Res* 90: 1108–1113, 2002.
364. Satoh T, Owada S, and Ishida M. Recent aspects in genetic renal mechanisms involved in hypertension. *Intern Med* 38: 919–926, 1999.
365. Sawa Y, Morishita R, Suzuki K, Kagisaki K, Kaneda Y, Maeda K, Kadoba K, and Matsuda H. A novel strategy for myocardial protection using in vivo transfection of cis element "decoy" against NFkappaB binding site: evidence for a role of NFkappaB in ischemia-reperfusion injury. *Circulation* 96: II280–II284, 1997.
366. 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.
367. Schindler TH, Hornig B, Buser PT, Olschewski M, Magosaki N, Pfisterer M, Nitzsche EU, Solzbach U, and Just H. Prognostic value of abnormal vasoreactivity of epicardial coronary arteries to sympathetic stimulation in patients with normal coronary angiograms. *Arterioscler Thromb Vasc Biol* 23: 495–501, 2003.
368. Schmidt AM, Hasu M, Popov D, Zhang JH, Chen J, Yan SD, Brett J, Kuwabara K, and Costache G. Receptor for advanced glycation end products (AGEs) has a central role in vessel wall interactions and gene activation in response to circulating AGE proteins. *Proc Natl Acad Sci U S A* 91: 8807–8811, 1994.
369. Schmidt AM, Hori O, Chen JX, Crandall J, Zhang J, Cao R, Yan SD, Brett J, and Stern D. Advanced glycation endproducts interacting with their endothelial receptor induce expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human endothelial cells and in mice: a potential mechanism for the accelerated vasculopathy of diabetes. *J Clin Invest* 96: 1395–1403, 1995.
370. Schmidt AM, Vianna M, Gerlach M, Brett J, Ryan J, Kao J, Esposito C, Hegarty H, Hurley W, and Clauss M. Isolation and characterization of two binding proteins for advanced glycosylation end products from bovine lung which are present on the endothelial cell surface. *J Biol Chem* 267: 14987–14997, 1992.
371. Schnyder G, Roffi M, Pin R, Flammer Y, Lange H, Eberli FR, Meier B, Turi ZG, and Hess OM. Decreased rate of coronary restenosis after lowering of plasma homocysteine levels. *N Engl J Med* 345: 1593–1600, 2001.
372. Shanmugam N, Reddy MA, Guha M, and Natarajan R. High glucose-induced expression of proinflammatory cytokine and chemokine genes in monocytic cells. *Diabetes* 52: 1256–1264, 2003.
373. Sharma R and Davidoff MN. Oxidative stress and endothelial dysfunction in heart failure. *Congest Heart Fail* 8: 165–172, 2002.
374. Shi GY, Hau JS, Wang SJ, Wu IS, Chang BI, Lin MT, Chow YH, Chang WC, Wing LY, and Jen CJ. Plasmin and the regulation of tissue-type plasminogen activator biosynthesis in human endothelial cells. *J Biol Chem* 267: 19363–19368, 1992.
375. Shimoda LA and Semenza GL. Functional analysis of the role of hypoxia-inducible factor 1 in the pathogenesis of hypoxic pulmonary hypertension. *Methods Enzymol* 381: 121–129, 2004.
376. Shimokawa H and Takeshita A. Rho-kinase is an important therapeutic target in cardiovascular medicine. *Arterioscler Thromb Vasc Biol* 25: 1767–1775, 2005.
377. Shimokawa H, Yasutake H, Fujii K, Owada MK, Nakaike R, Fukumoto Y, Takayanagi T, Nagao T, Egashira K, Fujishima M, and Takeshita A. The importance of the hyperpolarizing mechanism increases as the vessel size decreases in endothelium-dependent relaxations in rat mesenteric circulation. *J Cardiovasc Pharmacol* 28: 703–711, 1996.
378. Shinozaki K, Ayajiki K, Kashiwagi A, Masada M, and Okamura T. Malfunction of vascular control in lifestyle-related diseases: mechanisms underlying endothelial dysfunction in the insulin-resistant state. *J Pharmacol Sci* 96: 401–405, 2004.
379. Shiohira S, Yoshida T, Shirota S, Tsuchiya K, and Nitta K. Protective effect of carbon monoxide donor compounds in endotoxin-induced acute renal failure. *Am J Nephrol* 27: 441–446, 2007.
380. Sies H. Strategies of antioxidant defense. *Eur J Biochem* 215: 213–219, 1993.
381. Simkins S. Dinitrophenol and desiccated thyroid in the treatment of obesity. *JAMA* 108: 2210–2217, 1937.
382. Simon AR, Severgnini M, Takahashi S, Rozo L, Andrahbi B, Agyeman A, Cochran BH, Day RM, and Fanburg BL. 5-HT induction of c-fos gene expression requires reactive oxygen species and Rac1 and Ras GTPases. *Cell Biochem Biophys* 42: 263–276, 2005.
383. Singh R, Barden A, Mori T, and Beilin L. Advanced glycation end-products: a review. *Diabetologia* 44: 129–146, 2001.

384. Smith RAJ, Porteous CM, Coulter CV, and Murphy MP. Targeting and antioxidant to mitochondria. *Eur J Biochem* 263: 709–716, 1999.
385. Smith RM, McCarthy J, and Sack MN. TNF alpha is required for hypoxia-mediated right ventricular hypertrophy. *Mol Cell Biochem* 219: 139–143, 2001.
386. Sofola OA, Knill A, Hainsworth R, and Drinkhill M. Change in endothelial function in mesenteric arteries of Sprague-Dawley rats fed a high salt diet. *J Physiol* 543: 255–260, 2002.
387. Sorensen KE, Celermajer DS, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Thomas O, and Deanfield JE. Non-invasive measurement of human endothelium dependent arterial responses: accuracy and reproducibility. *Br Heart J* 74: 247–253, 1995.
388. Soulis JV, Farmakis TM, Giannoglou GD, and Louridas GE. Wall shear stress in normal left coronary artery tree. *J Biomech* 39: 742–749, 2006.
389. Soulis JV, Giannoglou GD, Chatzizisis YS, Farmakis TM, Giannakoulas GA, Parcharidis GE, and Louridas GE. Spatial and phasic oscillation of non-newtonian wall shear stress in human left coronary artery bifurcation: an insight to atherogenesis. *Coron Artery Dis* 17: 351–358, 2006.
390. Spence JD and Norris J. Infection, inflammation, and atherosclerosis. *Stroke* 34: 333–334, 2003.
391. Splaver A, Lamas GA, and Hennekens CH. Homocysteine and cardiovascular disease: biological mechanisms, observational epidemiology, and the need for randomized trials. *Am Heart J* 148: 34–40, 2004.
392. Stangl V, Gunther C, Jarrin A, Bramlage P, Moobed M, Staudt A, Baumann G, Stangl K, and Felix SB. Homocysteine inhibits TNF-alpha-induced endothelial adhesion molecule expression and monocyte adhesion via nuclear factor-kappaB dependent pathway. *Biochem Biophys Res Commun* 280: 1093–1100, 2001.
393. Stasch JP, Becker EM, Alonso-Alija C, Apeler H, Dembowsky K, Feurer A, Gerzer R, Minuth T, Perzborn E, Pleiss U, Schroder H, Schroeder W, Stahl E, Steinke W, Straub A, and Schramm M. NO-independent regulatory site on soluble guanylate cyclase. *Nature* 410: 212–215, 2001.
394. Steinberg D. Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. *Nat Med* 8: 1211–1217, 2002.
395. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, and Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction: implications for the syndrome of insulin resistance. *J Clin Invest* 97: 2601–2610, 1996.
396. Steiner MK, Preston IR, Klinger JR, and Hill NS. Pulmonary hypertension: inhaled nitric oxide, sildenafil and natriuretic peptides. *Curr Opin Pharmacol* 5: 245–250, 2005.
397. Steinhorn RH, Russell JA, Lakshminrusimha S, Gugino SF, Black SM, and Fineman JR. Altered endothelium-dependent relaxations in lambs with high pulmonary blood flow and pulmonary hypertension. *Am J Physiol* 280: H311–H317, 2001.
398. Steins MB, Padro T, Li CX, Mesters RM, Ostermann H, Hammel D, Scheld HH, Berdel WE, and Kienast J. Overexpression of tissue-type plasminogen activator in atherosclerotic human coronary arteries. *Atherosclerosis* 145: 173–180, 1999.
399. Stenvinkel P. Endothelial dysfunction and inflammation-is there a link? *Nephrol Dial Transplant* 16: 1968–1971, 2001.
400. Stephens NG, Parsons A, Schofield PM, Kelly F, Cheeseman K, and Mitchinson MJ. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). *Lancet* 347: 781–786, 1996.
401. Stone JR and Yang S. Hydrogen peroxide: a signaling messenger. *Antioxid Redox Signal* 8: 243–270, 2006.
402. Stone PH, Coskun AU, Yeghiazarians Y, Kinlay S, Popma JJ, Kuntz RE, and Feldman CL. Prediction of sites of coronary atherosclerosis progression: in vivo profiling of endothelial shear stress, lumen, and outer vessel wall characteristics to predict vascular behavior. *Curr Opin Cardiol* 18: 458–470, 2003.
403. Stuehr DJ, Santolini J, Wang ZQ, Wei CC, and Adak S. Update on mechanism and catalytic regulation in the NO synthases. *J Biol Chem* 279: 36167–36170, 2004.
404. Stühlinger MC, Tsao PS, Her JH, Kimoto M, Balint RF, and Cooke JP. Homocysteine impairs the nitric oxide synthase pathway: role of asymmetric dimethylarginine. *Circulation* 104: 2569–2575, 2001.
405. Sugiyama S, Okada Y, Sukhove GK, Virmani R, Heinecke JW, and Libby P. Macrophage myeloperoxidase regulation by granulocyte macrophage colony-stimulating factor in human atherosclerosis and implications in acute coronary syndromes. *Am J Pathol* 158: 879–891, 2001.
406. Sugiyama T, Yoshimoto T, Sato R, Fukai N, Ozawa N, Shichiri M, and Hirata Y. Endothelin-1 induces cyclooxygenase-1 expression and generation of reactive oxygen species in endothelial cells. *J Cardiovasc Pharmacol* 44(suppl 1): S332–S335, 2004.
407. Sullivan CC, Du L, Chu D, Cho AJ, Kido M, Wolf PL, Jamieson SW, and Thistlethwaite PA. Induction of pulmonary hypertension by an angiopoietin 1/TIE2/serotonin pathway. *Proc Natl Acad Sci U S A* 100: 12331–12336, 2003.
408. 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.
409. Szabo C and Ohshima H. DNA damage induced by peroxynitrite: subsequent biological effects. *Nitric Oxide* 1: 373–385, 1997.
410. Taddei S, Galetta F, Virdis A, Ghiadoni L, Salvetti G, Franzoni F, Giusti C, and Salvetti A. Physical activity prevents age-related impairment in nitric oxide availability in elderly athletes. *Circulation* 101: 2896–2901, 2000.
411. Tambyraja AL, Mitchell R, Driscoll PJ, Deans C, Parks RW, Rahman I, and Megson IL. Glutathione supplementation to University of Wisconsin solution causes endothelial dysfunction. *Transplant Immunol* 18: 146–150, 2007.
412. Tan KC, Chow WS, Ai VH, Metz C, Bucala R, and Lam KS. Advanced glycation end products and endothelial dysfunction in type 2 diabetes. *Diabetes Care* 25: 1055–1059, 2002.
413. Tanabe T, Maeda S, Miyauchi T, Iemitsu M, Takanashi M, Irukayama-Tomobe Y, Yokota T, Ohmori H, and Matsuda M. Exercise training improves ageing-induced decrease in eNOS expression of the aorta. *Acta Physiol Scand* 178: 3–10, 2003.
414. Targonski PV, Bonetti PO, Pumper GM, Higano ST, Holmes DR Jr, and Lerman A. Coronary endothelial dysfunction is associated with an increased risk of cerebrovascular events. *Circulation* 107: 2805–2809, 2003.
415. Teran FJ, Johnson RA, Stevenson BK, Peyton KJ, Jackson KE, Appleton SD, Durante W, and Johnson FK. Heme oxygenase-derived carbon monoxide promotes arteriolar endothelial dysfunction and contributes to salt-induced hy-

pertension in Dahl salt-sensitive rats. *Am J Physiol Regul Integr Comp Physiol* 288: R615–R622, 2005.
416. Thorup C, Jones CL, Gross SS, Moore LC, and Goligorsky MS. Carbon monoxide induces vasodilation and nitric oxide release but suppresses endothelial NOS. *Am J Physiol Renal Physiol* 277: F882–F889, 1999.
417. Title LM, Cummings PM, Giddens K, Genest JJ Jr, and Nassar BA. Effect of folic acid and antioxidant vitamins on endothelial dysfunction in patients with coronary artery disease. *J Am Coll Cardiol* 36: 758–765, 2000.
418. Toda N, Matsumoto T, and Yoshida K. Comparison of hypoxia-induced contraction in human, monkey, and dog coronary arteries. *Am J Physiol* 262: H678–H683, 1992.
419. Todd S, Woodward M, Tunstall-Pedoe H, and Bolton-Smith C. Dietary antioxidant vitamins and fiber in the etiology of cardiovascular disease and all-cause mortality: results from the Scottish Heart Health Study. *Am J Epidemiol* 150: 1073–1080, 1999.
420. Topal G, Brunet A, Millanvoye E, Boucher JL, Rendu F, Devynk MA, and David-dufilho M. Homocysteine induces oxidative stress by uncoupling of NO synthase activity through reduction of tetrahydrobiopterin. *Free Radic Biol Med* 36: 1532–1541, 2004.
421. Tornqvist H, Mills NL, Gonzalez M, Miller MR, Robinson SD, Megson IL, Macnee W, Donaldson K, Soderberg S, Newby DE, Sandstrom T, and Blomberg A. Persistent endothelial dysfunction following diesel exhaust inhalation in man. *Am J Respir Crit Care Med* 176: 395–400, 2007.
422. Tousoulis D, Antoniades C, Tentolouris C, Tsioufis C, Toutouza M, Toutouzas P, and Stefanadis C. Effects of combined administration of vitamins C and E on reactive hyperemia and inflammatory process in chronic smokers. *Atherosclerosis* 170: 261–267, 2003.
423. Tousoulis D, Davies G, Lefroy DC, Haider AW, and Crake T. Variable coronary vasomotor responses to acetylcholine in patients with normal coronary arteriograms: evidence for localised endothelial dysfunction. *Heart* 75: 261–266, 1996.
424. Touyz RM. Reactive oxygen species, vascular oxidative stress, and redox signaling in hypertension: what is the clinical significance? *Hypertension* 44: 248–252, 2004.
425. Touyz RM, Tabet F, and Schiffrin EL. Redox-dependent signalling by angiotensin II and vascular remodelling in hypertension. *Clin Exp Pharmacol Physiol* 30: 860–866, 2003.
426. Touyz RM, Yao G, Viel E, Amiri F, and Schiffrin EL. Angiotensin II and endothelin-1 regulate MAP kinases through different redox-dependent mechanisms in human vascular smooth muscle cells. *J Hypertens* 22: 1141–1149, 2004.
427. Traub O and Berk BC. Laminar shear stress: mechanisms by which endothelial cells transduce an atheroprotective force. *Arterioscler Thromb Vasc Biol* 18: 677–685, 1998.
428. Tricot O, Mallat Z, Heymes C, Belmin J, Leseche G, and Tedgui A. Relation between endothelial cell apoptosis and blood flow direction in human atherosclerotic plaques. *Circulation* 101: 2450–2453, 2000.
429. True AL, Olive M, Boehm M, San H, Westrick RJ, Raghavachari N, Xu X, Lynn EG, Sack MN, Munson PJ, Gladwin MT, and Nabel EG. Heme oxygenase-1 deficiency accelerates formation of arterial thrombosis through oxidative damage to the endothelium, which is rescued by inhaled carbon monoxide. *Circ Res* 101: 893–901, 2007.
430. Tschudi MR, Barton M, Bersinger NA, Moreau P, Cosentino F, Noll G, Malinski T, and Luscher TF. Effect of age on kinetics of nitric oxide release in rat aorta and pulmonary artery. *J Clin Invest* 98: 899–905, 1996.
431. Tuder RM, Cool CD, Geraci MW, Wang J, Abman SH, Wright L, Badesch D, and Voelkel NF. Prostacyclin synthase expression is decreased in lungs from patients with severe pulmonary hypertension. *Am J Respir Crit Care Med* 159: 1925–1932, 1999.
432. Tuder RM, Flook BE, and Voelkel NF. Increased gene expression for VEGF and the VEGF receptors KDR/Flk and Flt in lungs exposed to acute or to chronic hypoxia: modulation of gene expression by nitric oxide. *J Clin Invest* 95: 1798–807, 1995.
433. Uehata A, Takase B, Nishioka T, Kitamura K, Akima T, Kurita A, and Isojima K. Effect of quinapril *versus* nitrendipine on endothelial dysfunction in patients with systemic hypertension. *Am J Cardiol* 87: 1414–1416, 2001.
434. Urbich C and Dimmeler S. Endothelial progenitor cells: characterization and role in vascular biology. *Circ Res* 95: 343–353, 2004.
435. Valencia JV, Mone M, Zhang J, WEetall M, Buxton FP, and Hughes TE. Divergent pathways of gene expression are activated by the RAGE ligands S100b and AGE-BSA. *Diabetes* 53: 743–751, 2004.
436. Vallance P, Collier J, and Bhagat K. Infection, inflammation, and infarction: does acute endothelial dysfunction provide a link? *Lancet* 349: 1391–1392, 1997.
437. van der Loo B, Labugger R, Skepper JN, Bachschmid M, Kilo J, Powell JM, Palacios-Callender M, Erusalimsky JD, Quaschning T, Malinski T, Gygi D, Ullrich V, and Luscher TF. Enhanced peroxynitrite formation is associated with vascular aging. *J Exp Med* 192: 1731–1744, 2000.
438. van Hinsbergh VWM. Endothelial permeability for macromolecules: mechanistic aspects of pathophysiological modulation. *Arterioscler Thromb Vasc Biol* 17: 1018–1023, 1997.
439. Van Renterghem C, Vigne P, Barhanin J, Schmid-Alliana A, Frelin C, and Lazdunski M. Molecular mechanism of action of the vasoconstrictor peptide endothelin. *Biochem Biophys Res Commun* 157: 977–985, 1988.
440. van Venrooij FV, van de Ree MA, Bots ML, Stolk RP, Huisman MV, Banga JD, and DALI Study Group. Aggressive lipid lowering does not improve endothelial function in type 2 diabetes: the Diabetes Atorvastatin Lipid Intervention (DALI) Study: a randomized, double-blind, placebo-controlled trial. *Diabetes Care* 25: 1211–1216, 2002.
441. Vane JR, Bunting S, and Moncada S. Prostacyclin in physiology and pathophysiology. *Int Rev Exp Pathol* 23: 161–207, 1982.
442. Vasquez-Vivar J, Duquaine D, Whitsett J, Kalyanaraman B, and Rajagopalan S. Altered tetrahydrobiopterin metabolism in atherosclerosis: implications for use of oxidized tetrahydrobiopterin analogues and thiol antioxidants. *Arterioscler Thromb Vasc Biol* 22: 1655–1661, 2002.
443. Vasquez-Vivar J, Kalyanaraman B, and Kennedy MC. Mitochondrial aconitase is a source of hydroxyl radical: an electron spin resonance investigation. *J Biol Chem* 275: 14064–14069, 2000.
444. Verma S, Wang CH, Li SH, Dumont AS, Fedak PW, Badiwala MV, Dhillon B, Weisel RD, Li RK, Mickle DA, and Stewart DJ. A self-fulfilling prophecy: C-reactive protein attenuates nitric oxide production and inhibits angiogenesis. *Circulation* 106: 913–919, 2002.
445. Vermeulen EG, Stehouwer CD, Twisk JW, van den Berg M, de Jong SC, Mackaay AJ, van Campden CM, Visser FC, Jakobs CA, Bulterjis EJ, and Rauwerda JA. Effect of homocysteine-lowering treatment with folic acid plus vitamin B6 on progression of subclinical atherosclerosis: a randomised, placebo-controlled trial. *Lancet* 355: 517–522, 2000.

446. Vinten-Johansen J. Involvement of neutrophils in the pathogenesis of lethal myocardial reperfusion injury. *Cardiovasc Res* 61: 481–497, 2004.
447. Vlassara H, Brownlee M, Manogue KR, Dinarello CA, and Pasagian A. Cachetin, TNF and IL-1 induced by glucose-modified proteins: role in normal tissue remodelling. *Science* 240: 1546–1548, 1988.
448. Vlassara H, Fuh H, Makita Z, Krungkrai S, Cerami A, and Bucala R. Exogenous advanced glycosylation end products induce complex vasculat dysfunction in normal animals: a model for diabetic and aging complications. *Proc Natl Acad Sci U S A* 89: 12043–12047, 1992.
449. Vlassara H, Li YM, Imani F, Wojciechowicz D, Yang Z, Liu FT, and Cerami A. Identification of glaectin-3 as a high-affinity binding protein for advanced glycation end products (AGE): a new member of the AGE-receptor complex. *Mol Med* 1: 634–646, 1995.
450. Vlassara H and Palace MR. Diabetes and advanced glycation endproducts. *J Intern Med* 251: 87–101, 2002.
451. von Klot S, Peters A, Aalto P, Bellander T, Berglind N, D'Ippoliti D, Elosua R, Hormann A, Kulmala M, Lanki T, Lowel H, Pekkanen J, Picciotto S, Sunyer J, Forastiere F and health effects of particles on susceptible subpopulations (HEAPSS) study group: ambient air pollution is associated with increased risk of hospital cardiac readmissions of myocardial infarction survivors in five European cities. *Circulation* 112: 3073–3079, 2005.
452. Waid DK, Chell M, and El-Fakahany EE. M(2) and M(4) muscarinic subtypes couple to activation of endothelial nitric oxide synthase. *Pharmacology* 61: 37–42, 2000.
453. Walch L, Gascard JP, Dulmet E, Brink C, and Norel X. Evidence for a M_1 muscarinic receptor on the endothelium of human pulmonary veins. *Br J Pharmacol* 130: 73–78, 2000.
454. Wang D, Borrego-Conde LJ, Falck JR, Sharma KK, Wilcox CS, and Umans JG. Contributions of nitric oxide, EDHF, and EETs to endothelium-dependent relaxation in renal afferent arterioles. *Kidney Int* 63: 2187–2193, 2003.
455. Wang M, Zukas AM, Hui Y, Ricciotti E, Pure E, and FitzGerald GA. Deletion of microsomal prostaglandin E synthase-1 augments prostacyclin and retards atherogenesis. *Proc Natl Acad Sci U S A* 103: 14507–14512, 2006.
456. Wang R.Two's company, three's a crowd: can H2S be the third endogenous gaseous transmitter? *FASEB J* 16: 1792–1798, 2002.
457. Wang R, Wang Z, and Wu L. Carbon monoxide-induced vasorelaxation and the underlying mechanisms. *Br J Pharmacol* 121: 927–934, 1997.
458. Warabi E, Wada Y, Kajiwara H, Kobayashi M, Koshiba N, Hisada T, Shibata M, Ando J, Tsuchiya M, Kodama T, and Noguchi N. Effect on endothelial cell gene expression of shear stress, oxygen concentration, and low-density lipoprotein as studied by a novel flow cell culture system. *Free Radic Biol Med* 37: 682–694, 2004.
459. Wautier JL and Guillausseau PJ. Advanced glycation end products, their receptors and diabetic angiopathy. *Diabetes Metab* 27: 535–542, 2001.
460. Wedgwood S, McMullan DM, Bekker JM, Fineman JR, and Black SM. Role for endothelin-1-induced superoxide and peroxynitrite production in rebound pulmonary hypertension associated with inhaled nitric oxide therapy. *Circ Res* 89: 357–364, 2001.
461. West IC. Radicals and oxidative stress in diabetes. *Diabet Med* 17: 171–180, 2000.
462. Widlansky ME, Gokce N, Keaney JF Jr, and Vita JA. The clinical implications of endothelial dysfunction. *J Am Coll Cardiol* 42: 1149–1160, 2003.
463. Wilkens H, Guth A, Konig J, Forestier N, Cremers B, Hennen B, Bohm M, and Sybrecht GW. Effect of inhaled iloprost plus oral sildenafil in patients with primary pulmonary hypertension. *Circulation* 104: 1218–1222, 2001.
464. Wilkinson IB, Megson IL, MacCallum H, Rooijmans DF, Johnson SM, Boyd JL, Cockcroft JR, and Webb DJ. Acute methionine loading does not alter arterial stiffness in humans. *J Cardiovasc Pharmacol* 37: 1–5, 2001.
465. Williams FM. Neutrophils and myocardial reperfusion injury. *Pharmacol Ther* 72: 1–12, 1996.
466. Wilson SH, Simari RD, and Lerman A. The effect of endothelin-1 on nuclear factor kappa B in macrophages. *Biochem Biophys Res Commun* 286: 968–972, 2001.
467. Woo KS, Chook P, Lolin YI, Sanderson JE, Metreweli C, and Celermajer DS. Folic acid improves arterial endothelial function in adults with hyperhomocystinemia. *J Am Coll Cardiol* 34: 2002–2006, 1999.
468. Wu VY, Shearman CW, and Cohen MP. Identification of calnexin as a binding protein for Amadori-modified glycated albumin. *Biochem Biophys Res Commun* 284: 602–606, 2001.
469. Xiao J and Pang P. Does a general alteration in the nitric oxide synthesis system occur in spontaneously hypertensive rats? *Am J Physiol* 266: H272–H278, 1994.
470. Xu B, Chibber R, Ruggiero D, Kohner E, Ritter J, and Ferro A. Impairment of vascular endothelial nitric oxide synthase activity by advanced glycation end products. *FASEB J* 17: 1289–1291, 2003.
471. Xu B, Ji Y, Yao K, Cao YX, and Ferro A. Inhibition of human endothelial cell nitric oxide synthesis by advanced glycation end products but not glucose: relevance to diabetes. *Clin Sci* 109: 439–446, 2005.
472. Xu XP, Pollock JS, Tanner MA, and Myers PR. Hypoxia activates nitric oxide synthase and stimulates nitric oxide production in porcine coronary resistance arteriolar endothelial cells. *Cardiovasc Res* 30: 841–847, 1995.
473. Xu XP, Tanner MA, and Myers PR. Prostaglandin-mediated inhibition of nitric oxide production by bovine aortic endothelium during hypoxia. *Cardiovasc Res* 30: 345–350, 1995.
474. Yamakura F, Taka H, Fujimura T, and Murayama K. Inactivation of human manganese-superoxide dismutase by peroxynitrite is caused by exclusive nitration of tyrosine 34 to 3-nitrotyrosine. *J Biol Chem* 273: 14085–14089, 1998.
475. Yamawaki H and Iwai N. Mechanisms underlying nanosized air-pollution-mediated progression of atherosclerosis: carbon black causes cytotoxic injury/inflammation and inhibits cell growth in vascular endothelial cells. *Circ J* 70: 129–140, 2006.
476. Yan SD, Schmidt AM, Anderson GM, Zhang J, Brett J, Zou YS, Pinsky D, and Stern D. Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. *J Biol Chem* 269: 9889–9897, 1994.
477. Yang BC and Mehta JL. Critical role of endothelium in sustained arterial contraction during prolonged hypoxia. *Am J Physiol* 268: H1015–H1020, 1995.
478. Yang Z, Makita Z, Horii Y, Brunelle S, Cerami A, Sehajpal P, Suthanthiran M, and Vlassara H. Two novel rat lever membrane proteins that bind advanced glycosylation endproducts: relationship to macrophage receptor for glucose-modified proteins. *J Exp Med* 174: 515–524, 1991.
479. Yeh CH, Sturgis L, Haidacher J, Zhang XN, Sherwood SJ, Bjercke RJ, Juhasz O, Crow MT, Tilton RG, and Denner L. Requirement for p38 and p44-p42 mitogen-activated protein kinases in RAGE-mediated nuclear factor-kappaB tran-

scriptional activation and cytokine secretion. *Diabetes* 50: 1495–1504, 2001.
480. You J, Golding EM, and Bryan RM Jr. Arachidonic acid metabolites, hydrogen peroxide, and EDHF in cerebral arteries. *Am J Physiol Heart Circ Physiol* 289: H1077–H1083, 2005.
481. Yu SM, Hung LM, and Lin CC. cGMP-elevating agents suppress proliferation of vascular smooth muscle cells by inhibiting the activation of epidermal growth factor signaling pathway. *Circulation* 95: 1269–1277, 1997.
482. Yusuf S, Dagnenais G, Pogue J, Bosch J, and Sleight P. Vitamin E supplementation and cardiovascular events in high-risk patients: the Heart Outcomes Prevention Evaluation Study Investigators. *N Engl J Med* 342: 154–160, 2000.
483. Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, and Dagenais G. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients: the Heart Outcomes Prevention Evaluation Study Investigators. *N Engl J Med* 342: 145–153, 2000.
484. Zamora MA, Dempsey EC, Walchak SJ, and Stelzner TJ. BQ123, an ETA receptor antagonist, inhibits endothelin-1-mediated proliferation of human pulmonary artery smooth muscle cells. *Am J Respir Cell Mol Biol* 9: 429–433, 1993.
485. Zeiher AM, Drexler H, Wollschlager H, and Just H. Endothelial dysfunction of the coronary microvasculature is associated with coronary blood flow regulation in patients with early atherosclerosis. *Circulation* 84: 1984–1992, 1991.
486. Zeiher AM, Goebel H, Schachinger V, and Ihling C. Tissue endothelin-1 immunoreactivity in the active coronary atherosclerotic plaque: a clue to the mechanism of increased vasoreactivity of the culprit lesion in unstable angina. *Circulation* 91: 941–947, 1995.
487. Zelis R and Flaim SF. Alterations in vasomotor tone in congestive heart failure. *Prog Cardiovasc Dis* 24: 437–459, 1982.
488. Zhang C, Xu X, Potter BJ, Wang W, Kuo L, Michael L, Bagby GJ, and Chilian WM. TNF-alpha contributes to endothelial dysfunction in ischemia/reperfusion injury. *Arterioscler Thromb Vasc Biol* 26: 475–480, 2006.
489. Zhang R, Brennan ML, Fu X, Aviles RJ, Pearce GL, Penn MS, Topol EJ, Sprecher DL, and Hazen SL. Association between myeloperoxidase levels and risk of coronary artery disease. *JAMA* 286: 2136–2142, 2001.
490. Zhang R, Brennan ML, Shen Z, MacPherson JC, Schmitt D, Molenda CE, and Hazen SL. Myeloperoxidase functions as a major enzymatic catalyst for initiation of lipid peroxidation at sites of inflammation. *J Biol Chem* 277: 46116–46122, 2002.
491. Zhang R, Shen Z, Nauseef WM, and Hazen SL. Defects in leukocyte-mediated initiation of lipid peroxidation in plasma as studied in myeloperoxidase-deficient subjects: systematic identification of multiple endogenous diffusible substrates for myeloperoxidase in plasma. *Blood* 99: 1802–1810, 2002.
492. Zhang Y, Park TS, and Gidday JM. Hypoxic preconditioning protects human brain endothelium from ischemic apoptosis by Akt-dependent survivin activation. *Am J Physiol Heart Circ Physiol* 292: H2573–H2581, 2007.
493. Zhao W and Wang R. H_2S-induced vasorelaxation and underlying cellular and molecular mechanisms. *Am J Physiol Heart Circ Physiol* 283: H474–H480, 2002.
494. Zhao W, Zhang J, Lu Y, and Wang R. Modulation of endogenous production of H_2S in rat tissues. *Can J Physiol Pharmacol* 81: 848–853, 2003.
495. Zhao W, Zhang J, Lu Y, and Wang R. The vasorelaxant effect of H_2S as a novel endogenous gaseous K_{ATP} channel opener. *EMBO J* 20: 6000–6016, 2001.
496. Zheng L, Nukuna B, Brennan ML, Sun M, Goormastic M, Settle M, Schmitt D, Fu X, Thomson L, Fox PL, Ischiropoulos H, Smith JD, Kinter M, and Hazen SL. Apolipoprotein A-I is a selective target for myeloperoxidase-catalyzed oxidation and functional impairment in subjects with cardiovascular disease. *J Clin Invest* 114: 529–541, 2004.
497. Zou MH. Peroxynitrite and protein tyrosine nitration of prostacyclin synthase. *Prostaglandins Other Lipid Mediat* 82: 119–127, 2007.

Address reprint requests to:
Prof. Ian L Megson
Free Radical Research Facility, Department of Diabetes
UHI Millennium Institute
The Green House, Beechwood Business Park North
Inverness, UK IV2 3BL

E-mail: ian.megson@uhi.ac.uk

Date of first submission to ARS Central, December 10, 2007; date of final revised submission, March 18, 2008; date of acceptance, March 18, 2008.

This article has been cited by:

1. A. T. Treweeke, T. J. Winterburn, I. Mackenzie, F. Barrett, C. Barr, G. F. Rushworth, I. Dransfield, S. M. MacRury, I. L. Megson. 2012. N-Acetylcysteine inhibits platelet–monocyte conjugation in patients with type 2 diabetes with depleted intraplatelet glutathione: a randomised controlled trial. *Diabetologia* **55**:11, 2920-2928. [CrossRef]
2. Chad S. Weldy, Ian P. Luttrell, Collin C. White, Vicki Morgan-Stevenson, Theo K. Bammler, Richard P. Beyer, Zahra Afsharinejad, Francis Kim, Kanchan Chitaley, Terrance J. Kavanagh. 2012. Glutathione (GSH) and the GSH synthesis gene Gclm modulate vascular reactivity in mice. *Free Radical Biology and Medicine* **53**:6, 1264-1278. [CrossRef]
3. Saibal Biswas, Ian Megson, Catherine Shaw, Irfan RahmanCigarette Smoking, Inflammation, and Obesity 85-112. [CrossRef]
4. Ignacio Ricci-Cabello, Manuel Olalla Herrera, Reyes Artacho. 2012. Possible role of milk-derived bioactive peptides in the treatment and prevention of metabolic syndrome. *Nutrition Reviews* no-no. [CrossRef]
5. Laura Reyes, Ronald Garcia, Silvia Ruiz, Mahshid Dehghan, Patricio López-Jaramillo. 2012. Nutritional status among women with pre-eclampsia and healthy pregnant and non-pregnant women in a Latin American country. *Journal of Obstetrics and Gynaecology Research* no-no. [CrossRef]
6. James M. McCabe, John R. Teerlink. 2012. Bile Salts for the Treatment of Heart Failure. *Journal of the American College of Cardiology* **59**:6, 593-594. [CrossRef]
7. Basma Basha, Samson Mathews Samuel, Chris R. Triggle, Hong Ding. 2012. Endothelial Dysfunction in Diabetes Mellitus: Possible Involvement of Endoplasmic Reticulum Stress?. *Experimental Diabetes Research* **2012**, 1-14. [CrossRef]
8. 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]
9. Robert J. Weiss, Elijah Saunders, Mark Greathouse. 2011. Efficacy and Tolerability of Nebivolol in Stage I–II Hypertension: A Pooled Analysis of Data From Three Randomized, Placebo-Controlled Monotherapy Trials. *Clinical Therapeutics* . [CrossRef]
10. Hong Wang, Yue-Jin Yang, Hai-Yan Qian, Qian Zhang, Hui Xu, Jian-Jun Li. 2011. Resveratrol in cardiovascular disease: what is known from current research?. *Heart Failure Reviews* . [CrossRef]
11. Henriette Frikke-Schmidt, Martin Roursgaard, Jens Lykkesfeldt, Steffen Loft, Jacob Klenø Nøjgaard, Peter Møller. 2011. Effect of vitamin C and iron chelation on diesel exhaust particle and carbon black induced oxidative damage and cell adhesion molecule expression in human endothelial cells. *Toxicology Letters* **203**:3, 181-189. [CrossRef]
12. Mo Yin Mok, Chak Sing Lau, Sonny Sau Hin Chiu, Annette Wai Kwan Tso, Yi Lo, Lawrence Siu Chun Law, Ka Fung Mak, Woon Sing Wong, Peh Lan Khong, Karen Siu Ling Lam. 2011. Systemic sclerosis is an independent risk factor for increased coronary artery calcium deposition. *Arthritis & Rheumatism* **63**:5, 1387-1395. [CrossRef]
13. C. McGuinnes, R. Duffin, S. Brown, N. L. Mills, I. L. Megson, W. MacNee, S. Johnston, S. L. Lu, L. Tran, R. Li, X. Wang, D. E. Newby, K. Donaldson. 2011. Surface Derivatization State of Polystyrene Latex Nanoparticles Determines both Their Potency and Their Mechanism of Causing Human Platelet Aggregation In Vitro. *Toxicological Sciences* **119**:2, 359-368. [CrossRef]
14. Kyle R Gibson, Tim J Winterburn, Fiona Barrett, Sushma Sharma, Sandra M MacRury, Ian L Megson. 2011. Therapeutic potential of N-acetylcysteine as an antiplatelet agent in patients with type-2 diabetes. *Cardiovascular Diabetology* **10**:1, 43. [CrossRef]
15. Rowan Flynn, Joshua M Buckler, Chongren Tang, Francis Kim, David A Dichek. 2010. Helper-dependent Adenoviral Vectors are Superior In Vitro to First-generation Vectors for Endothelial Cell-targeted Gene Therapy. *Molecular Therapy* **18**:12, 2121-2129. [CrossRef]
16. C. Venkata S. Ram. 2010. Beta-Blockers in Hypertension. *The American Journal of Cardiology* **106**:12, 1819-1825. [CrossRef]
17. MY Mok, SSH Chiu, Y Lo, HKF Mak, WS Wong, PL Khong, CS Lau. 2010. Authors' Reply. *Scandinavian Journal of Rheumatology* **39**:6, 526-526. [CrossRef]
18. Ick-Mo Chung, Young-Myeong Kim, Mi-Hyun Yoo, Mi-Kyung Shin, Chun-Ki Kim, Suk Hyo Suh. 2010. Immobilization stress induces endothelial dysfunction by oxidative stress via the activation of the angiotensin II/its type I receptor pathway. *Atherosclerosis* **213**:1, 109-114. [CrossRef]
19. Sanjali Verma, Krishna Reddy, Pitchai Balakumar. 2010. The Defensive Effect of Benfotiamine in Sodium Arsenite-Induced Experimental Vascular Endothelial Dysfunction. *Biological Trace Element Research* **137**:1, 96-109. [CrossRef]

20. Mo Yin Mok, Chak Sing Lau. 2010. The burden and measurement of cardiovascular disease in SSc. *Nature Reviews Rheumatology* **6**:7, 430-434. [CrossRef]
21. Song Qu, Eric N. Liberda, Qingshan Qu, Lung-Chi Chen. 2010. In Vitro Assessment of the Inflammatory Response of Respiratory Endothelial Cells Exposed to Particulate Matter. *Journal of Toxicology and Environmental Health, Part A* **73**:16, 1113-1121. [CrossRef]
22. Milad S. Bitar, Adel K. Ayed, Samy M. Abdel-Halim, Esma R. Isenovic, Fahd Al-Mulla. 2010. Inflammation and apoptosis in aortic tissues of aged type II diabetes: Amelioration with #-lipoic acid through phosphatidylinositol 3-kinase/Akt- dependent mechanism. *Life Sciences* **86**:23-24, 844-853. [CrossRef]
23. Gnanapragasam Arunachalam, Hongwei Yao, Isaac K. Sundar, Samuel Caito, Irfan Rahman. 2010. SIRT1 regulates oxidant- and cigarette smoke-induced eNOS acetylation in endothelial cells: Role of resveratrol. *Biochemical and Biophysical Research Communications* **393**:1, 66-72. [CrossRef]
24. K. Stangl, V. Stangl. 2010. The ubiquitin-proteasome pathway and endothelial (dys)function. *Cardiovascular Research* **85**:2, 281-290. [CrossRef]
25. Ana R L Ludke, Francisca Mosele, Rafaela Caron-Lienert, Maria Flávia Ribeiro, Wânia Partata, Susana Llesuy, Alex Sander Araujo, Pawan Singal, Adriane Belló-Klein. 2010. Modulation of Monocrotaline-Induced Cor Pulmonale by Grape Juice. *Journal of Cardiovascular Pharmacology* **55**:1, 89-95. [CrossRef]
26. Saad Ahmad, Francesca Cesana, Edward Lamperti, Haralambos Gavras, Jun Yu. 2009. Attenuation of Angiotensin II–Induced Hypertension and Cardiac Hypertrophy in Transgenic Mice Overexpressing a Type 1 Receptor Mutant. *American Journal of Hypertension* **22**:12, 1320-1325. [CrossRef]
27. Luciana Hannibal, Alla Glushchenko, Donald JacobsenFolate and Vascular Disease 291-323. [CrossRef]
28. 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]
29. 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]
30. Arshad Rahman , Fabeha Fazal . 2009. Hug Tightly and Say Goodbye: Role of Endothelial ICAM-1 in Leukocyte Transmigration. *Antioxidants & Redox Signaling* **11**:4, 823-839. [Abstract] [Full Text PDF] [Full Text PDF with Links]
31. Andrea Salonia. 2009. Is ED Still Only Equal to ED?. *European Urology* **55**:4, 794-797. [CrossRef]
32. Yin Ruixing, Wu Jinzhen, Lin Weixiong, Chen Yuming, Yang Dezhai, Pan Shangling. 2009. The environmental and genetic evidence for the association of hyperlipidemia and hypertension. *Journal of Hypertension* **27**:2, 251-258. [CrossRef]
33. SALLY METSUYANIM, RAN LEVY, MIRIAM DAVIDOVITS, BENJAMIN DEKEL. 2009. Molecular Evaluation of Circulating Endothelial Progenitor Cells in Children Undergoing Hemodialysis and After Kidney Transplantation. *Pediatric Research* **65**:2, 221-225. [CrossRef]