Endothelial Dysfunction
— The First Step Toward Coronary Arteriosclerosis —

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The endothelium causes relaxations of the underlying vascular smooth muscle, by releasing nitric oxide (NO). The endothelial cells also can evoke hyperpolarization of the vascular smooth muscle cells (endothelium-dependent hyperpolarizations, endothelium-derived hyperpolarizing factors-mediated responses). Endothelium-dependent relaxations involve both pertussis toxin-sensitive Gi and pertussis toxin-insensitive Gq coupling proteins. The endothelial release of NO is reduced in diabetes and hypertension. Arteries covered with regenerated endothelium lose the pertussis-toxin sensitive pathway for NO-release. This dysfunction favors vasospasm, thrombosis, penetration of macrophages, cellular growth and the inflammatory reaction leading to atherosclerosis. Endothelial cells also release endothelium-derived contracting factors (EDCF). Most endothelium-dependent contractions are mediated by vasoconstrictor prostanoids (endoperoxides and prostacyclin), which activate thromboxane-prostanoid (TP)-receptors of the underlying vascular smooth muscle cells. EDCF-mediated responses are augmented by aging, hypertension and diabetes. Thus, endothelial dysfunction is the first step toward coronary arteriosclerosis. (Circ J 2009; 73: 595–601)

Key Words: Arteriosclerosis; Endothelium; Endothelium-derived relaxing factors; Vasodilatation

T he removal of the endothelium prevents the relaxations of isolated arteries to acetylcholine1 because the endothelial cells release vasodilator substance (s), termed endothelium-derived relaxing factors (EDRF) (Figure 1). The EDRF described by Furchgott and Zawadzki1 activates soluble guanylyl cyclase in the vascular smooth muscle cells, increasing the formation of cyclic guanosine monophosphate and is destroyed by superoxide anions.2–3 It has been identified as nitric oxide (NO) (Figure 1).4–6 Besides NO, other endothelium-derived hyperpolarizing factors (EDHF) can cause hyperpolarizations of the underlying vascular smooth muscle.7–9 Endothelial cells also can release endothelium-derived contracting factors (EDCF).2,3,10,11 When the release of NO and EDHFs is reduced and the production of EDCFs is enhanced, endothelial dysfunction ensues. This review briefly summarizes earlier more exhaustive reviews, to which the reader is referred for details and references to original experimental work, and focuses on the role of the imbalance between the production of NO and that of EDCFs in the genesis of vascular disease.12–20

NO

Protective Role

Besides acetylcholine, a number of more physiological stimuli [physical forces, circulating hormones (catecholamines, vasopressin, aldosterone), platelet products (serotonin, adenosine diphosphate (ADP), autacoids (histamine, bradykinin), and prostaglandins] can evoke endothelium-dependent relaxations due solely or in part to the release of NO by the endothelial cells (Figure 2). The gaseous mediator plays a key role in the protective role exerted by the endothelium against coronary disease. It is produced by the Ca2+-dependent, constitutive isoform of NO synthase (eNOS, NOS III) (Figure 1).6,21–25 NO prevents abnormal constriction (vasospasm) of the coronary arteries, and inhibits the aggregation of platelets, the expression of endothelial adhesion molecules, and thus the adhesion and penetration of macrophages. NO also inhibits both the release and the action of endothelin-1 (Figure 3). When the protective role of NO is hampered, the inflammatory response26 that leads to atherosclerosis can be set in motion.27,18,17,12 Hence, endothelial dysfunction has become a hallmark, and indeed a predictor of cardiovascular disease.

The ability of the endothelial cells to respond to thrombin and platelet products by releasing NO has been demonstrated both in isolated arteries and the intact organism. Thus, isolated coronary arteries with a normal endothelium relax when exposed to thrombin or aggregating platelets. Serotonin (5-hydroxytryptamine, 5-HT) and, to a lesser extent, ADP are the mediators released by platelets that can activate eNOS. Serotonin stimulates endothelial 5-HT1D serotonergic receptors while ADP activates P2y purinoceptors (Figure 3). The 5-HT1D receptors and those for thrombin are coupled to eNOS through pertussis toxin-sensitive Gi-proteins, while the P2y purinoceptors are linked to the enzyme by Gq-proteins. Thus, if platelet aggregation were to occur in a healthy coronary artery, the platelet-derived serotonin (and ADP) and the locally produced thrombin will stimulate the endothelial cells to release NO, which will relax the underlying smooth muscle, thus increasing blood flow and mechanically impeding the progression of the coagulation process. NO, in synergy with prostacyclin, also exerts a feedback inhibition on platelet aggregation. In the
Figure 1. A schematic of the possible mechanisms by which the production of nitric oxide (NO) is regulated in endothelial cells. NO is produced through the enzymatic conversion of L-arginine by NO synthase (endothelial or type III, eNOS). The transcription of this enzyme is regulated genomically by hormones and growth factors. Stability of eNOS mRNA is modulated by statins and hormones. eNOS enzyme activity requires calcium, calmodulin, nicotinamide adenine dinucleotide phosphate (NADPH), and 5, 6, 7, 8-tetrahydrobiopterine (BH$_4$). Enzyme activity is regulated by complexing to these proteins in microdomains of the endothelial cell. Association with this complex of heat shock protein 90 (HSP 90) increases enzyme activity. Stimulation of specific receptors on the endothelial surface (R) complexed with guanine nucleotide regulatory proteins, which are sensitive to pertussis toxin (Gi) or insensitive to pertussis toxin (Gq), activate intracellular pathways that modulate eNOS activity post-translationally through HSP 90 or AKT-phosphorylation. Association of eNOS with caveolin-1 or glycosylation of the enzyme reduces activity. A metabolite of L-arginine, asymmetric dimethyl arginine (ADMA), decreases production of the NO through competitive binding to eNOS. Thus, this endogenous amine might be a risk factor for the development of cardiovascular disease. +, indicates stimulation; −, indicates inhibition; ?, indicates those pathways in which the regulation is unknown (reproduced from Reference 18 with permission).

Figure 2. Some of the neurohumoral mediators that cause the release of endothelium-derived relaxing factors (EDRF) through activation of specific endothelial receptors (circles). AA, arachidonic acid; ACh, acetylcholine; ADP, adenosine diphosphate; a, a adrenergic receptor; AVP, arginine vasopressin; B, kinin receptor; E, epinephrine; ET, endothelin, endothelin-receptor; H, histaminergic receptor; 5-HT, serotonin (5-hydroxytryptamine), serotoninergic receptor; M, muscarinic receptor; NE, norepinephrine; P, purinergic receptor; PAR(?), thrombin receptor; VEGF, vascular endothelial growth factor; VP, vasopressin receptor (reproduced from Reference 18 with permission).
traumatic absence of the endothelium, or if the endothelial cells become dysfunctional, the release of serotonin and thromboxane A2 by the platelets is not impeded and they cause contraction of the vascular smooth muscle, initiating the vascular phase of hemostasis. The protective, endothelium-dependent responses to aggregating platelets (and thrombin) are most prominent in coronary and cerebral arteries.

**Chronic Modulation**

**Upregulation**

Acute and chronic increases in the shear stress exerted by the blood on the endothelial cells augment the release of EDRF/NO (Figure 2)\(^{28,29}\). The acute effect of increases in shear stress on NO release underlies flow-mediated dilatation, a response used in humans to estimate the functional state of the endothelium. The chronic effect of shear stress causing upregulation of eNOS and greater activation (phosphorylation) of the enzyme explains the beneficial effects of regular exercise on endothelial function\(^{18,25}\).

Physiological levels of estrogens augment endothelium-dependent relaxations and accelerate endothelial healing after injury. This potentiating effect of estrogens involves both genomic (Figure 1) and non-genomic effects\(^{15,30}\). Phytoestrogens and selective estrogen receptor modulators also potentiate endothelium-dependent relaxations/vasodilations. It is likely that this potentiating effect of estrogens on NO release helps to explain why endothelium-dependent relaxations are more pronounced in arteries from female than male animals [and thus why women are protected against coronary disease, at least until the age of menopause]\(^{18}\).

The chronic dietary intake of ω3-unsaturated fatty acids potentiates the endothelium-dependent relaxations of coronary arteries to aggregating platelets and other stimuli, and has anti-atherogenic properties. This is also the case for flavonoids and other polyphenols, present in red wine, green tea or dark chocolate\(^{18}\).

**Downregulation**

The enzymes in endothelial cells that can produce superoxide, from which other oxygen-derived free radicals are derived, are anions and include NADPH oxidase, xanthine oxidase, cyclooxygenase and eNOS itself, when it is uncoupled by the lack of substrate (L-arginine) or a shortage of the essential co-factor, tetrahydrobiopterin (BH\(_4\))\(^{11}\). Superoxide anions can be dismutated by superoxide dismutase (SOD) to hydrogen peroxide (H\(_2\)O\(_2\)), which acts as an EDHF and participates in endothelium-dependent relaxations\(^{15,17,32,33}\) or is broken down by catalase. Superoxide anions also scavenge NO avidly with the resulting formation of peroxynitrite. This reduces the bioavailability of NO\(^{18}\). Hence, increases in oxidative stress are associated with reduced endothelium-dependent relaxations, and antioxidants can improve such responses in vitro and in vivo\(^{33}\). Aging reduces endothelium-dependent vasodilations both in vitro and in vivo\(^{15,18}\). This is due to the reduced release and bioavailability of NO. However, an important part of the endothelial dysfunction with aging is due to the endothelial release of vasoconstrictor prostaglandins (see below). Active and passive smoking and chronic exposure to air pollution also blunt endothelium-dependent vasodilations\(^{34}\).

In both animals and humans, hypercholesterolemia reduces endothelium-dependent relaxations/dilatations and the normalization of the cholesterol levels with treatment restores the response. Obese animals and humans exhibit reduced responses to endothelium-dependent vasodilators. Weight loss alone or exercise training can improve endothelium-dependent responses\(^{18}\).

**Vascular Disease**

Endothelium-dependent relaxations are reduced in isolated arteries from different animal models of hypertension and the response to endothelium-dependent vasodilators is also blunted in hypertensive humans. This blunting can be corrected by an appropriate treatment. It might reflect the premature aging of the vasculature exposed chronically to the increased arterial blood pressure\(^{18}\). In the spontaneously hypertensive rat, the blunting of endothelium-dependent relaxations/vasodilations is due mainly to the concomitant release of endothelium-derived vasoconstrictor prostaglandins (see below) rather than to a reduced release of NO.

Insulin resistance and diabetes cause an impairment of arterial endothelium-dependent relaxations in animals and...
Humans, presumably due to the chronic exposure to hyperglycemia. In addition to a reduced release and bioavailability of NO, the production of vasoconstrictor prostanoids contributes importantly to the endothelial dysfunction of diabetes (see below).

Individuals at increased risk of coronary heart disease exhibit impaired dilatations in response to endothelium-dependent stimuli both in the coronary and peripheral circulations. In both animals and humans, the presence of endothelial dysfunction predicts the severity of the outcome, in particular the occurrence of myocardial infarction and stroke.

Endothelium-dependent relaxations are reduced in coronary and peripheral arteries of animals and humans with ventricular hypertrophy and/or heart failure. The degree of impairment of endothelium-dependent vasodilatations predicts the outcome in such patients.

**Regenerated Endothelium and Atherosclerosis**

The endothelial turnover is accelerated by cardiovascular risk factors including hypertension and diabetes. The apoptotic cells are removed by the blood stream and are replaced by regenerated endothelium formed by neighboring cells freed of contact inhibition, and/or circulating endothelial progenitor cells. However, the regenerated endothelium is dysfunctional (Figure 3). Thus, 1 month after in vivo balloon denudation of the endothelium of part of the artery, despite total relining of the endothelial surface, rings covered with regenerated endothelium exhibit reduced relaxations to aggregating platelets, serotonin or thrombin.

By contrast, relaxations evoked by ADP and bradykinin, which both depend on the Gq-signaling cascade, as well as those to the calcium ionophore, A23187, are normal, illustrating the ability of the regenerated endothelial cells to produce NO. These observations demonstrate a selective dysfunction of the Gi-dependent responses in regenerated endothelial cells. This selective dysfunction can be reduced by the chronic intake of α3 unsaturated fatty acid but is exacerbated by a chronic hypercholesterolemic diet, which results in typical accelerated atherosclerotic lesions in the area of previous denudation. Thus, the selective dysfunction of regenerated endothelial cells appears to be the first step toward the atherosclerotic process.

Primary cultures derived from regenerated endothelial cells had the appearance and markers of accelerated senescence, a reduced expression and activity of eNOS, a greater production of oxygen-derived free radicals, increased uptake of modified low-density lipoprotein cholesterol (LDL) and generated more oxidized LDL (oxyLDL). By contrast, the immunohistochemical presence of Gi-proteins appeared normal. The genomic changes observed in cultures of regenerated endothelial cells were consistent with those phenotypic and functional changes. As increased extracellular concentrations of oxyLDL reduce the production of EDRF/NO and the endothelium-dependent relaxations to serotonin, it is logical to conclude that an augmented presence of the modified lipid is the cause of the selective loss in Gi-protein mediated responses in regenerated endothelial (Figure 3).

Obviously, this is not the only negative effect of oxygen-derived free radicals and oxyLDL that plays a central role in the atherosclerotic process. The greater production of superoxide anions will reduce the bioavailability of NO and increase the levels of peroxynitrite.

Whatever the cause is of their dysfunction, the endothelial cells cannot produce enough NO in response to platelets and thrombin, and this NO deficiency permits the inflammatory reaction leading to atherosclerosis.

**Endothelium-Derived Vasoconstrictor Prostanoids**

The endothelial cells can initiate contractions of the underlying vascular smooth muscle cells, and diffusible substances are responsible for these endothelium-dependent increases in tone. The up-to-date evidence demonstrates that the vasoconstrictor prostanoids mediate most endothelium-dependent contractions, demonstrated in response to acetylcholine and other vasoactive substances in blood vessels from different species.

![Figure 4](image-url)
Pivotal Role of Cyclooxygenase

Endothelium-dependent contractions are prevented by non-selective inhibitors of cyclooxygenase, demonstrating the key role of this enzyme in the phenomenon.

Bioassay studies indicate that the vasoconstrictor prostanoids involved are produced by cyclooxygenase in the endothelium rather than in the vascular smooth muscle. Functional studies in arteries of the spontaneously hypertensive rat (SHR) using preferential and selective inhibitors of the 2 isoforms of the enzyme [constitutive cyclooxygenase-1 (COX-1) and inducible cyclooxygenase-2 (COX-2)], as well as molecular biology experiments and data obtained in the aorta of genetically modified mice concur to suggest that COX-1 is the major source of EDCF.

The release of EDCF is usually initiated by vasoactive mediators acting in the endothelial cell membrane (Figure 4). These include acetylcholine (activating endothelial M3-muscarinic receptors) and ADP (activating purinoceptors). Endothelium-dependent contractions are reduced by decreasing the extracellular Ca\(^{2+}\)-concentration and by vitamin D derivatives, and can be triggered by the calcium ionophore, A23187, and are paralleled by an increase in endothelial cytosolic Ca\(^{2+}\)-concentration. Thus, an increase in intracellular Ca\(^{2+}\)-concentration is the initial trigger for endothelium-dependent contractions, presumably by activating phospholipase A\(_2\), which then makes arachidonic acid available to the endothelial cyclooxygenase setting, which in turn produces the vasoconstrictor prostanoids (Figure 4).

Cyclooxygenase transforms arachidonic acid into endoperoxides and these prostaglandin precursors are released during endothelium-dependent contractions. The endoperoxides, per se, can activate vascular smooth muscle and act as an EDCF.

Endoperoxides are converted into prostacyclin, thromboxane A\(_2\), prostaglandin D\(_2\), prostaglandin E\(_2\) and/or prostaglandin F\(_2\)α by selective synthases. Of the latter, the gene expression of prostacyclin synthase is the most abundant in endothelial cells. During endothelium-dependent contractions, the release of prostacyclin outweighs that of other prostaglandins. In arteries where endothelium-dependent contractions to acetylcholine are prominent, prostacyclin no longer evokes the expected relaxation/dilatation. Thus, it seems logical to conclude that endoperoxides and prostacyclin are the major mediators of endothelium-dependent contractions to acetylcholine.

The cyclooxygenase-dependent, endothelium-dependent contractions are inhibited by antagonists of thromboxane-prostanoid (TP) receptors. The TP-receptors involved are those of the vascular smooth muscle that initiate the contractile response.

Modulation of EDCF-Mediated Responses

Inhibitors of NO synthase cause an immediate poteniation, whereas previous exposure to NO, whether released from the endothelial cells or added exogenously, causes a long-term inhibition of endothelium-dependent contractions. These observations imply that conditions resulting in a lesser bioavailability of NO favor the occurrence of EDCF-mediated contractions/constrictions. In some arteries, SOD abolishes endothelium-dependent contractions suggesting that superoxide anions act as an intercellular messenger. In other blood vessels, however, SOD does not affect endothelium-dependent contractions while cell-permeable scavengers of superoxide anions depress the
response. Acetylcholine and A23187 cause a burst of endothelial free radical production that is prevented by indomethacin. Thus, cyclooxygenase appears to be the main source of superoxide anions during endothelium-dependent contractions, and their production is not a primary event. Once produced, the free radicals amplify the EDCF-mediated response, presumably in part by stimulating cyclooxygenase of the vascular smooth muscle. The oxygen-derived free radicals might reach the vascular smooth muscle cells through the myoendothelial gap junctions. Obviously, the scavenging action of superoxide anions on NO, by reducing the bioavailability of the latter, favors the occurrence of endothelium-dependent contractions.

Endothelium-dependent contractions increase with aging. Inhibitors of cyclooxygenase and TP-receptor antagonists prevent or revert the blunting of endothelium-dependent relaxations/vasodilatations caused by aging. The age-dependency of the response is explained best by an increased oxidative stress resulting in the upregulation of COX-1 and/or the induction of COX-2. In addition, although the gene expression of prostacyclin synthase augments with age, the prostanoid no longer evokes relaxations in arteries from aging animals. In humans, indomethacin augments the relaxations to acetylcholine in isolated arteries of older patients as well as the vasodilator response to the muscarinic agonist in the forearm of aging people, suggesting that the importance of EDCF-mediated responses also increases with age in that species.

Vascular Disease

The endothelium-dependent relaxations to acetylcholine are blunted by hypertension (see above) and endothelium-dependent contractions to acetylcholine are more pronounced in arteries of the SHR than in those of normotensive Wistar–Kyoto rats (WKY). These changes are prevented by inhibitors of cyclooxygenase and antagonists at the TP-receptors. The increase in intracellular endothelial Ca2+-concentration caused by acetylcholine is greater in SHR than in WKY arteries, while during exposure to A23187, it is comparable, suggesting that a key aspect of the prominence of endothelium-dependent contractions in hypertension is due to an abnormal endothelial handling of calcium (Figure 5). In addition, in the aorta of hypertensive strain, the expression/presence of COX-1 is increased but this overexpression is not present in arteries of pre-hypertensive SHR. These findings suggest that the overexpression of the enzyme in arteries from adult hypertensive animals reflects premature aging of the endothelium rather than a genetic predisposition. The gene expression of prostacyclin synthase is more abundant in endothelial cells of SHR than of WKY, and the protein presence of the enzyme is augmented by hypertension. These changes explain why acetylcholine causes a greater release of endoperoxides and prostacyclin in SHR than in WKY arteries. The occurrence of endothelium-dependent contractions is facilitated also by the fact that prostacyclin no longer causes relaxations in arteries of hypertensive animals. Obviously, the absence of a vasodilator response to prostacyclin contributes, and helps to explain why in humans cardiovascular disease is accelerated by a dysfunctional prostacyclin receptor mutation. In addition, although the mRNA expression and protein presence of TP receptors are comparable in arteries of WKY and SHR, the latter are hyper-responsive to the vasoconstrictor effect of endoperoxides and prostacyclin, a hyper-responsiveness already present in pre-hypertensive animals.

It is thus not a consequence of premature aging following the chronic exposure to an increased arterial blood pressure, and constitutes one of the genetic platforms of the disease. Because aspirin and indomethacin potentiate the vasodilator response to acetylcholine in the forearm of patients with hypertension but not in that of normotensive subjects, EDCF-mediated responses also are part of the endothelial dysfunction of human hypertension.

Endothelium-dependent relaxations to acetylcholine are blunted in arteries from animals with diabetes, but this is due in part to the concomitant release of EDCF. This can be attributed to the chronic exposure of the endothelial cells to high glucose, resulting in increased oxidative stress and overexpression of both COX-1 and COX-2.

Conclusion

Healthy endothelial cells respond to a number of stimuli (eg, serotonin from aggregating platelets and thrombin) by releasing NO, which relaxes the underlying vascular smooth muscle. NO, together with prostacyclin, inhibits platelet aggregation. It also reduces the endothelial expression of adhesion molecules and thus the adhesion and penetration of leukocytes (macrophages). Endothelium-derived NO also prevents the proliferation of vascular smooth muscle cells and limits the formation of oxLDL. Aging and certain lifestyle factors (eg, lack of exercise, Western diet, pollution and smoking), or certain diseases (eg, diabetes and hypertension) result in a lesser release of NO and an acceleration of the turnover of the apoptotic process in the endothelium. The apoptotic cells are replaced by regenerated endothelium. However, such regenerated cells are senescent, and incapable of reducing the required amounts of NO, which facilitates the inflammatory response leading to the formation of atherosclerotic plaques. A shortage of NO also unleashes the production of endothelium-derived vasoconstrictor prostanoids (EDCF), in particular endoperoxides and prostacyclin. These prostanoids activate TP-receptors of the vascular smooth muscle leading to vasoconstriction that further augments the endothelial dysfunction.

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References


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