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Editorial

Endothelial dysfunction

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Endothelial dysfunction is defined as an abnormal endothelial response leading to a reduction in the bioavailability of nitric oxide, and impaired vasodilatation [1]. It has been found to be associated with several disorders of the cardiovascular system, including diabetes, hypertension, hyperlipidemia, and heart failure, and vascular risk factors of cigarette smoking [2,3].

The vascular endothelium acts as a 'plasma–tissue barrier' and has a crucial role in controlling vascular function, with the balance between endothelium-derived vasodilators and vasoconstrictors determining vascular tone and the pathophysiological consequences [4]. In addition, the reduction in nitric oxide bioavailability can adversely affect platelet aggregation, vascular wall inflammation, and smooth muscle cell proliferation.

The clinical consequences of endothelial dysfunction include the development of atherosclerosis, acute coronary syndromes, cardiac failure, and erectile dysfunction. It is no wonder that the vascular endothelium is the focus of so much attention, when it is now recognized that a defect in the nitric oxide–cyclic guanosine 3'5'-monophosphate system in smooth muscle cells before the development of overt cardiovascular disease in men with erectile dysfunction is an early marker of systemic vascular abnormalities [3,5].

Measures of endothelial dysfunction have been shown to be improved by drugs that benefit cardiovascular morbidity and mortality (angiotensin-converting-enzyme inhibitors in cardiac failure; statins and angiotensin-converting enzyme inhibitors in ischemic heart disease), and erectile dysfunction, heart failure, and diabetes (phosphodiesterase type 5 inhibitors) [6].

As a number of clinical conditions are clearly related to endothelial dysfunction, it becomes increasingly important to develop and validate means of its evaluation, and subsequently to determine whether improving endothelial dysfunction may in turn improve the long-term clinical outcome of conditions such as diabetes and cardiac failure.

The endothelium has a pivotal role through regulating vascular homeostasis. Once believed to be an inert monolayer of cells simply lining blood vessels, the endothelium is now recognized to have the most important role, in local regulation of vessel function. As our understanding of endothelial cell biology has developed – an increasingly rapid awakening – we have come to recognize its worrying potential to give rise to vascular diseases, with the important positive implications that we could use endothelial progenitor cells to promote new vessel formation, and gene therapy to modify endothelial vascular function. There is promise and potential, and it is timely to review what we know and how to look forward – these are exciting times for preventative strategies.

This issue of *Heart and Metabolism* reviews what is known about endothelial dysfunction, from the basics to its clinical expression and treatment, with an examination of the imaging of coronary endothelial dysfunction by positron emission tomography. Disease mechanisms are open to modification and the next months and (few) years offer important opportunities for endothelial cell research and treatment. Over the past 5 years, we have come to realize that erectile dysfunction is determined by endothelial dysfunction, and that it is modified by phosphodiesterase type 5 inhibitors, which improve endothelial dysfunction by acting within the smooth muscle cell [3,6].

By looking beyond the box of cardiovascular presentations, we may have opportunities to modify disease progression at a very early stage – which is why I feel this issue of *Heart and Metabolism* will form a template for new ideas in this exciting area. ■

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Endothelial function and dysfunction

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Abstract

The endothelium mediates a number of responses (relaxations or contractions) of isolated arteries and veins from animals and humans. The endothelium-dependent relaxation results from the release by the endothelial cells of potent nonprostanoid vasodilator substances. Among these, the best characterized is endothelium-derived relaxing factor (EDRF), which most probably is nitric oxide. Nitric oxide is formed by the metabolism of L-arginine by the constitutive nitric oxide synthase of endothelial cells. In arterial smooth muscle, the relaxation evoked by nitric oxide is best explained by the stimulation by nitric oxide of soluble guanylate cyclase, which leads to the accumulation of cyclic 3'5'-guanosine monophosphate. The endothelial cells also release prostacyclin and a substance that causes hyperpolarization of the cell membrane (endothelium-derived hyperpolarizing factor, EDHF). The release of relaxing factors can be initiated by circulating hormones (catecholamines, vasopressin, oxytocin, and estrogens). The release of EDRF from the endothelium can be mediated by both pertussis toxin-sensitive (α_2-adrenergic activation, serotonin, aggregating platelets, leukotrienes) and pertussis toxin-insensitive (adenosine diphosphate, bradykinin) G proteins. In blood vessels from animals with regenerated and reperfused endothelium, or atherosclerosis, or both, there is a selective loss of the pertussis toxin sensitive mechanisms of EDRF release that favors the occurrence of vasospasm, thrombosis, and cellular growth.
■ *Heart Metab.* 2004;22:5–10.

Keywords: Nitric oxide, atherosclerosis, platelet aggregation, G proteins, endothelium-dependent relaxation

Introduction

In 1980, Furchgott and Zawadzki [1] demonstrated that endothelial cells have an obligatory role in the relaxation of isolated arteries in response to acetylcholine. This pivotal observation has revolutionized thinking about the local control of vasomotor function. The endothelium-dependent responses are caused by the release of several diffusible substances (endothelium-derived relaxing [EDRF] and contracting factors) from the endothelial cells. This review briefly summarizes the observations, obtained mainly in the author's laboratory, that have examined how the production of relaxing factors by endothelial cells underlies moment-to-moment changes in the tone of the surrounding vascular smooth muscle cells, and how a lack of this function by endothelial cells eventually initiates atherosclerosis and thus vascular disease. It updates similar, more exhaustive overviews [2–13].

Endothelium-derived relaxing factors

Endothelium-derived nitric oxide

The short-lived diffusible factor that underlies endothelium-dependent relaxation in response to acetylcholine [1] has been identified as nitric oxide. Endothelial nitric oxide is formed from the guanidine-nitrogen terminal of L-arginine by the action of endothelial constitutive nitric oxide synthase (nitric oxide synthase III, eNOS). The activation of eNOS depends on the intracellular concentration of calcium ions in the endothelial cells, and is Ca^{2+}-calmodulin-dependent (*Figure 1*). The activity of the enzyme requires cofactors: in particular, reduced

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nicotinamide adenine dinucleotide phosphate, and 5,6,7,8-tetrahydrobiopterin. eNOS can be inhibited competitively by synthetic L-arginine analogs such as N^G-monomethyl-L-arginine or N^G-nitro-L-arginine, or by the endogenous inhibitor, asymmetric dimethyl arginine. Nitric oxide diffuses to the underlying smooth muscle cells and, in them, stimulates cytosolic soluble guanylate cylase, which accelerates the formation of cyclic 3′5′-guanosine monophosphate (cyclic GMP). The cyclic nucleotide in turn inhibits the contractile process. Nitric oxide is the major contributor to endothelium-dependent relaxation in large arteries [1–15]. In the intact organism, both animal and human, the inhibitors of nitric oxide synthase cause vasoconstriction in most vascular beds and an increase in systemic arterial pressure, not only because they prevent the direct inhibitory action of nitric oxide on the vascular smooth muscle, but also because nitric oxide inhibits the production of renin and of endothelin 1 [16].

Nitric oxide is also released in the lumen of the blood vessel. Because it is scavenged by the oxyhemoglobin of the blood, it does not fulfil a hormonal role. However, at the interface between the blood and the blood vessel wall, it inhibits the adhesion of platelets and white cells to the endothelium. It acts (in strong synergy with prostacyclin) to inhibit platelet aggregation [3,4,9,15]. It also inhibits the growth of the vascular smooth muscle cells and prevents the production of adhesion molecules [17] (*Figure 2*).

The activity of eNOS can be upregulated acutely. For example, the shear forces exerted by the flowing
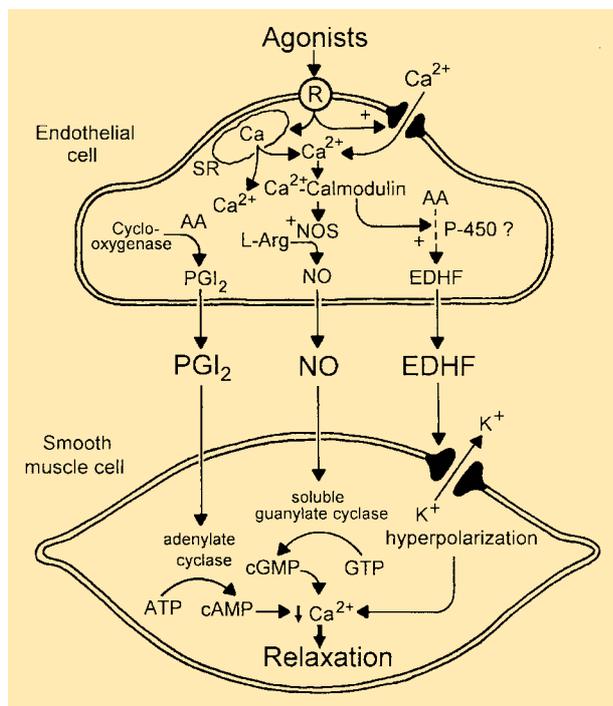

Figure 1. Role of the increase in cytosolic calcium concentration in the release of endothelium-derived relaxing factor(s). Endothelial receptor activation induces an influx of calcium into the cytoplasm of the endothelial cell; after interaction with calmodulin, this activates nitric oxide synthase (NOS) and cyclooxygenase, and leads to the release of endothelium-derived hyperpolarizing factor (EDHF). Nitric oxide (NO) causes relaxation by activating the formation of cyclic 3′5′-guanosine monophosphate (cGMP) from guanosine triphosphate (GTP). EDHF causes hyperpolarization and relaxation by opening potassium (K^+) channels. Prostacyclin (PGI_2) causes relaxation by activating adenylate cyclase, which leads to the formation of cyclic adenosine monophosphate (cAMP). Any increase in cytosolic calcium (including that induced by the calcium ionophore, A23187) causes the release of relaxing factors. When agonists activate the endothelial cells, an increase in inositol phosphate may contribute to the increase in cytoplasmic Ca^{2+} by releasing it from the sarcoplasmic reticulum (SR). AA, arachidonic acid; L-Arg, L-arginine; P-450, cytochrome P-450; R, membrane receptor. (From Vanhoutte et al [42], with permission.)
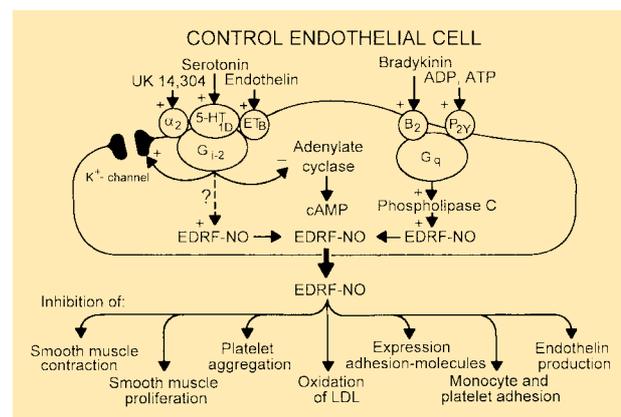
Figure 2. Postulated signal transduction processes in an endothelial cell. Activation of the cell causes the release of endothelium-derived relaxing factor nitric oxide (EDRF-NO), which has important protective effects in the vascular wall. α, α-adrenergic; B, bradykinin receptor; cAMP, cyclic AMP; ET, endothelin receptors; G, coupling proteins; 5-HT, serotonin (5-hydroxytryptamine) receptor; P, purinoceptor. (From Vanhoutte [11], with permission.)

blood on the endothelial cells are one of the main regulators of the local release of nitric oxide, a mechanism that explains flow dependent vasodilation. Several substances, whether circulating in the blood or produced by the blood vessel wall, can increase the release of nitric oxide through activation of specific receptors on the endothelial cell membrane (*Figure 3*). They include hormones (eg, estrogen, catecholamines, vasopressin), neurotransmitters (eg, substance P), autacoids (bradykinin, histamine), and products formed during platelet aggregation (serotonin, adenosine diphosphate [ADP] or blood coagulation (thrombin). The cell membrane receptors for these substances are coupled to the activation of eNOS by two different families of G proteins (*Figure 2*). Thus, in coronary arteries, α_2-adrenergic receptors, serotonin receptors, and thrombin receptors are coupled to pertussis toxin-sensitive Gi proteins, whereas, in contrast, the receptors for ADP or

bradykinin are not coupled to the production of nitric oxide by pertussis-toxin sensitive G proteins [18]. The activation of eNOS by bradykinin involves low molecular weight G proteins of the Rho family [19]. In coronary and cerebral arteries, aggregating platelets induce endothelium-dependent relaxation, and the presence of a healthy endothelium inhibits the constriction induced by the platelet products (thromboxane A_2 and serotonin). Serotonin, acting on 5-HT1D serotonin receptors, plays the major part in this response, whereas ADP, activating P2y-purinoceptors, contributes little (*Figure 2*). The release of nitric oxide, both toward the underlying smooth muscle and at the interface with the blood, in response to thrombin and platelet-derived serotonin is pivotal for the protective role played by the healthy endothelium against the platelet attack (*Figure 4*) [3,8,13].

Prostacyclin

Prostacyclin, formed primarily in endothelial cells, relaxes vascular smooth muscle by stimulation of adenylate cyclase, with a resulting increased production of cyclic 3'5'-adenosine monophosphate (cyclic AMP). It acts synergistically with nitric oxide to inhibit platelet aggregation (*Figure 4*) [3,6,14].

Endothelium-dependent hyperpolarizing factor

In large and small arteries from different species (including the human), acetylcholine, and other endothelium-dependent vasodilators, cause endothelium-dependent hyperpolarization which can contribute to endothelium-dependent relaxation. The hyperpolarization has been attributed to a diffusible endothelium-derived hyperpolarizing factor (EDHF) different from nitric oxide and prostacyclin, although these last two can, in certain but not all blood vessels, cause hyperpolarization of vascular smooth muscle. The exact nature of EDHF remains a matter of intense debate. Among the more recent candidates to explain endothelium-dependent hyperpolarization, gap junctions, epoxyeicosatrienoic acids, potassium ions, and hydrogen peroxide appear to have major roles [20–23] (*Figure 1*).

The contribution of hyperpolarization to endothelium-dependent relaxation varies as a function of the
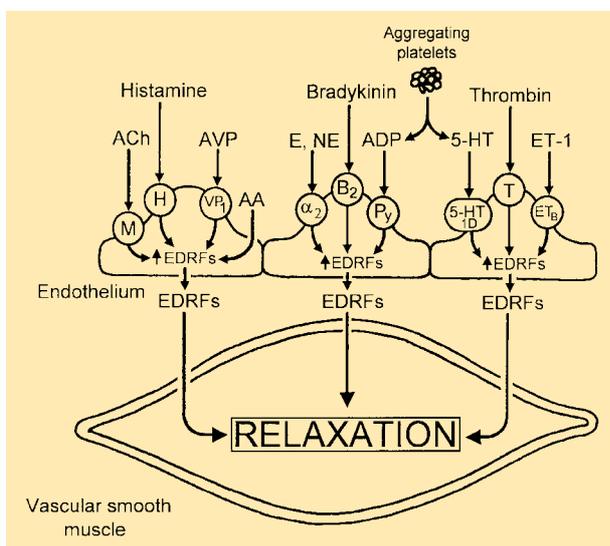
Figure 3. Some of the neurohumoral mediators that cause the release of endothelium-derived relaxing factors (EDRFs) through activation of specific endothelial receptors (encircled). α, α-adrenergic receptor; A, adrenaline (epinephrine); AA, arachidonic acid; Ach, acetylcholine; ADP, adenosine diphosphate; AVP, arginine vasopressin; B, kinin receptor; E, estrogen; ET, endothelin, endothelin-receptor; H, histaminergic receptor; 5-HT, serotonin (5-hydroxytryptamine), serotoninergic receptor; M, muscarinic receptor; NA, noradrenaline (norepinephrine); P, purinergic receptor; T, thrombin receptor; VP, vasopressinergic receptor. (From Vanhoutte [11], with permission.)

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Figure 4. Interaction between platelet products, thrombin, and endothelium. If the endothelium is intact, several of the substances released from the platelets [in particular, the adenine nucleotides (ADP and AT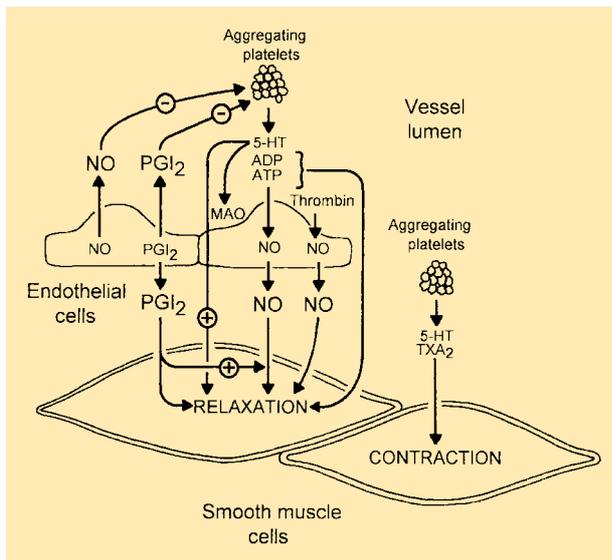P) and serotonin (5-hydroxytryptamine, 5-HT)] cause the release of endothelium-derived relaxing factor (EDRF) and prostacyclin (PGI_2). The same is true for any thrombin formed. The released EDRF will relax the underlying vascular smooth muscle, opening up the blood vessel, and thus flushing the microaggregate away; it will also be released towards the lumen of the blood vessel to brake platelet adhesion to the endothelium and, synergistically with prostacyclin, inhibit platelet aggregation. In addition, monoamine oxidase (MAO) and other enzymes will break down the vasoconstrictor serotonin, limiting the amount of the monoamine that can diffuse toward the smooth muscle. Finally, the endothelium acts as a physical barrier that prevents the access to the smooth muscle of the vasoconstrictor platelet products serotonin and thromboxane A_2 (TXA_2). These different functions of the endothelium have a key role in preventing unwanted coagulation and vasospastic episodes in blood vessels with a normal intima. If the endothelial cells are removed (eg, by trauma), the protective role of the endothelium is lost locally, platelets can adhere and aggregate, and vasoconstriction follows; this contributes to the vascular phase of hemostasis. +, activation; −, inhibition; NO, nitric oxide. (From Vanhoutte [11], with permission.)

size of the blood vessel and thus is more pronounced in smaller than in larger arteries [23,24]. In the latter, although both mediators can contribute to endothelium-dependent relaxation, nitric oxide predominates under normal circumstances. However, in these large arteries, such as the coronaries, EDHF can maintain near normal endothelium-dependent relaxation when the synthesis of nitric oxide is dysfunctional [25]. In certain cases, nitric oxide exerts an inhibitory effect on endothelium-dependent hyperpolarization [26].

Chronic modulation

Chronic modulatory influences that can upregulate the release of relaxing factors by endothelial cells include chronic increases in blood flow, exercise training, estrogen administration, and intake of ω_3-unsaturated fatty acids, red wine polyphenols, green tea, and other antioxidants [27–30].

Endothelial dysfunction

In the course of aging, and in several types of vascular disease and hypertension, the endothelial cells become dysfunctional [3,4,8,10–13]. This dysfunction is evident as an impairment of endothelium-dependent relaxation, mainly as the result of a reduced release of EDRFs, in particular nitric oxide, although production of endothelium-derived vasoconstrictor substances may contribute [2,3,5,31,32].

Regenerated endothelium

The normal aging process induces a turnover (apoptotic death, desquamation followed by regeneration) of endothelial cells. Unfortunately, regenerated endothelial cells have lost part of the ability to release nitric oxide in response to platelet aggregation [33,34], because they respond minimally to serotonin and other substances using the Gi protein-dependent pathway controlling the release of nitric oxide (*Figure 2*); the Gi proteins are present, but exhibit a reduced activity [35–39]. The loss of the pertussis toxin sensitive response is selective, and it does not apply, at least initially, to endothelium-dependent responses mediated by Gq-coupling proteins, in particular that to bradykinin [37,38]. It is caused by the greater accumulation of oxidized low density lipoproteins by the regenerated endothelial cells [40,41]. The reduced release of nitric oxide can be compensated in part by the larger contribution of EDHF to the endothelium-dependent relaxation [25].

Hypercholesterolemia and atherosclerosis

Hypercholesterolemia impairs endothelium-dependent relaxation [33,34]. In contrast, endothelium-independent relaxation in response to exogenous nitric oxide remains largely normal. In the initial phase of the atherosclerotic process, endothelial dysfunction is limited to the pertussis toxin sensitive, Gi protein-dependent pathway (*Figure 2*). Thus the ability of regenerated endothelial cells, chronically exposed to high cholesterol concentrations, to ADP-ribosylate pertussis toxin is reduced [39]. Hence, in coronary arteries from hypercholesterolemic pigs, endothelium-dependent relaxation in response to serotonin, α_2-adrenergic agonists, aggregating platelets, or thrombin is depressed, whereas those induced by ADP and bradykinin are maintained [33–39]. Oxidized low-density lipoprotein induces, in vitro, a similar selective endothelial dysfunction, whereas at higher concentrations it also inhibits endothelium-dependent relaxation in response to stimuli that are not Gi protein-dependent [40] (*Figure 2*).

Summary

The most important aspect of endothelial dysfunction is the reduced release or bioavailibility of nitric oxide, which probably is the fundamental, initial step of the atherosclerotic process. This hypothesis implies that aging and prolonged exposure to shear stress, coupled with risk factors such as obesity, diabetes, high blood pressure, and smoking, accelerate endothelial turnover and endothelial regeneration. Thus larger and larger sections of the endothelial lining (particularly in areas of turbulence) can no longer prevent platelet adhesion and aggregation, and become insensitive to thrombin. The negative feedback that nitric oxide, together with prostacyclin, exerts on platelet aggregation decreases steadily, whereas vasoconstrictor and growth-promoting substances (serotonin and thromboxane A_2) are released in increasing amounts, together with growth factors such as platelet-derived growth factor. This sequence of events permits the local inflammatory response and initiates the characteristic morphological changes in atherosclerosis, in particular because the local shortage of nitric oxide unleashes the growth process [3,7–14]. ■

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Clinical expression of endothelial dysfunction

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Abstract

Endothelial dysfunction is a composite risk score of conventional cardiovascular risk factors and novel risk factors. It can be readily measured by failure of the endothelium to promote vasodilatation in response to acetylcholine and reactive hyperemia. In patients with coronary artery disease, it is predictive of myocardial infarction. Measurements of forearm endothelial function, while less invasive than those of those of coronary endothelial function, correlate closely with them. Endothelial dysfunction has implications in many cardiovascular diseases. In hypertension and carotid stenosis, forearm endothelial function is independently associated with cardiovascular events and cerebral ischemic events, respectively. In congestive heart failure, endothelial dysfunction is an early disease marker. High concentrations of low-density lipoprotein and triglycerides, low concentrations of high-density lipoprotein, obesity, and smoking are all associated with endothelial dysfunction. In healthy individuals, the presence of endothelial dysfunction could be a marker of genetic predeposition to hypertension and myocardial infarction. Endothelial dysfunction has also been used as a surrogate marker to measure the therapeutic response to various risk-modifying treatments. Thus assessment of endothelial dysfunction may represent a rational approach for risk assessment of patients with or at risk for cardiovascular diseases. This review highlights important concepts from recent clinical studies that focused on endothelial dysfunction in patients.
■ *Heart Metab.* 2004;22:11–16.

Keywords: Endothelial dysfunction, nitric oxide, cardiovascular disease, endothelium, forearm endothelial, coronary artery disease

Introduction

The endothelium functions to regulate vascular tone by releasing endothelium-derived relaxing (EDRF) and contracting factors. One key relaxing factor is nitric oxide, a free radical signaling gas that promotes vasodilatation, inhibits platelet aggregation, and may thereby inhibit the development of atherosclerosis and arterial thrombosis. Endothelial dysfunction can be readily measured by failure of the endothelium to promote vasodilatation through nitric oxide.

Cardiovascular risk factors commonly lead to a cascade of events that impair endothelial function. Therefore, endothelial dysfunction serves as an integrated index of damage caused by cardiovascular risk factors. Multivariate analysis has shown that it is in fact a composite risk score of conventional risk factors [1,2].

This review will highlight the relevance of endothelial dysfunction to a number of vascular syndromes.

Measuring coronary endothelial dysfunction

The testing of coronary endothelial dysfunction was described by Ludmer et al in 1986 [3]. Acetylcholine is infused into the left anterior descending artery. The coronary artery diameter can be measured with quantitative coronary angiography, and coronary blood flow can be measured with intracoronary flow Doppler. Endothelium-independent responses are induced by administration of nitroglycerin, which directly promotes vasodilatation by acting on smooth muscles. Endothelium-dependent responses are induced by acetylcholine, which promotes the release of nitric oxide from the endothelium, thereby

promoting smooth muscle relaxation; this vasodilatation is therefore said to be endothelium-dependent. Without a functional endothelium, acetylcholine causes vasoconstriction in vascular smooth muscle. Thus, with a functional endothelium, administration of acetylcholine results in vasodilatation and an increase in coronary blood flow, whereas with endothelial dysfunction the acetylcholine-mediated vasodilatation and increase in coronary blood flow are attenuated.

Clinical application in coronary artery disease

Since Ludmer's description of the technique, the findings of several studies [4–10] have supported the concept that measurement of coronary endothelial dysfunction may provide a prognosticator for coronary artery disease (*Table I*). Al Suwaidi et al [4] conducted a 24-month follow-up study of patients with mild coronary artery disease. The study population consisted of 157 patients with angiographically identified coronary artery lesions, less than 40% stenosis, and no evidence of coronary spasm. Patients were divided into three groups: normal endothelial function, mild endothelial dysfunction, and severe endothelial dysfunction. Interestingly, the distribution of cardiovascular risk factors (eg, age, sex, diabetes mellitus, hypertension, hypercholesterolemia, smoking) was similar between the three groups. Noninvasive studies, including treadmill exercise testing, exercise thallium, and exercise echo cardiograms,

Table I. Clinical studies assessing endothelial dysfunction in vascular disease.

Reference	Participants	Follow-up	Measurement	Conclusion
Suwaidi et al [4]	157 patients with mildly diseased coronary arteries	28 months	Response to infusing Ach into coronary artery	Only severe endothelial dysfunction led to cardiac events (14%)
Schächinger et al [5]	121 patients undergoing either catheterization for chest pain evaluation or PTCA for single-vessel disease	7.7 years	Coronary Ach infusion, cold pressor testing and flow-dependent dilatation; vasodilatation vs vasodilation response	Vasoconstrictor response associated with increased cardiovascular death, unstable angina, MI, need for coronary or peripheral revascularization, ischemic stroke
Halcox et al [6]	132 patients had angiographically identified CAD; 176 patients had angiographically normal coronary artery	46 months	Coronary Ach infusion, change in coronary vascular resistance and epicardial diameter	Independent predictors of cardiovascular death, acute MI, unstable angina pectoris, and acute ischemic stroke
Neunteufl et al [7]	73 patients with chest pain	5 years	Forearm FMD after reactive hyperemia	FMD < 10%: 50% experience MI or need for revascularization in 5 years FMD > 10%: these outcomes in only 15%
Heitzer et al [8]	281 patients with angiographically documented CAD	45 months	Forearm blood flow after Ach infusion	Ach-induced vasodilatation independently associated with death from cardiovascular cause, MI, ischemic stroke, need for cardiac and peripheral revascularization
Perticone et al [9]	225 never-treated hypertensive patients	31.5 months	Forearm blood flow after Ach infusion	Predicts future cardiac, cerebrovascular, or peripheral vascular event
Hung Yi Hsu et al [10]	58 patients with carotid stenosis > 50%	Cross-sectional	Forearm FMD after reactive hyperemia	Impaired forearm FMD associated with symptomatic carotid stenosis

Ach, acetylcholine; CAD, coronary artery disease; FMD, flow-mediated dilatation; MI, myocardial infarction; PTCA, percutaneous transluminal coronary angioplasty.

were also similar with respect to the prevalence of positive results. Quantitative coronary ultrasound revealed similar prevalences of plaque. During 28 months of follow-up, only patients in the severe endothelial dysfunction group developed cardiac events [4]. Schächinger et al [5] conducted a 7.7-year follow-up study in 147 patients with angiographic evidence of coronary artery disease, and reported similar results. Hasdai et al [11] have also shown that severe coronary endothelial dysfunction is associated with myocardial perfusion defects. Halcox et al [6] generalized the patient population to include those with normal coronary arteries. In their study of 308 patients undergoing coronary catheterization, 132 had coronary artery disease identified by angiography, whereas 176 patients had angiographically normal coronary arteries. These patients were followed for 46 months for cardiovascular events. Although regression analysis showed no significant interaction between coronary artery disease and coronary endothelial dysfunction, in multivariate analysis coronary endothelial function, age, coronary artery disease, and body mass index were all independent risk factors for cardiovascular events [6]. These studies suggest that severe endothelial dysfunction is an independent risk factor for cardiac events in patients with nonobstructive coronary artery disease.

In the Mayo Clinic, more than 700 measurements of coronary endothelial function have been made since 1992. The indication for making these measurements is the finding of a normal coronary artery by angiogram in patients who exhibit symptoms of angina: endothelial dysfunction is found in a significant percentage of these individuals. Normal and abnormal tracings of intracoronary Doppler flow velocity in response to adenosine, obtained for the evaluation of coronary flow reserve, are shown in *Figure 1*.

Measuring forearm flow mediated vasodilatation

Despite its prognostic value, measurement of coronary endothelial function is very invasive. In the 1990s, less invasive ultrasonographic measurement of brachial artery endothelial function was developed, in line with evidence that endothelial dysfunction in peripheral vessels correlates well with that in the coronary artery [12,13]. An ultrasound system
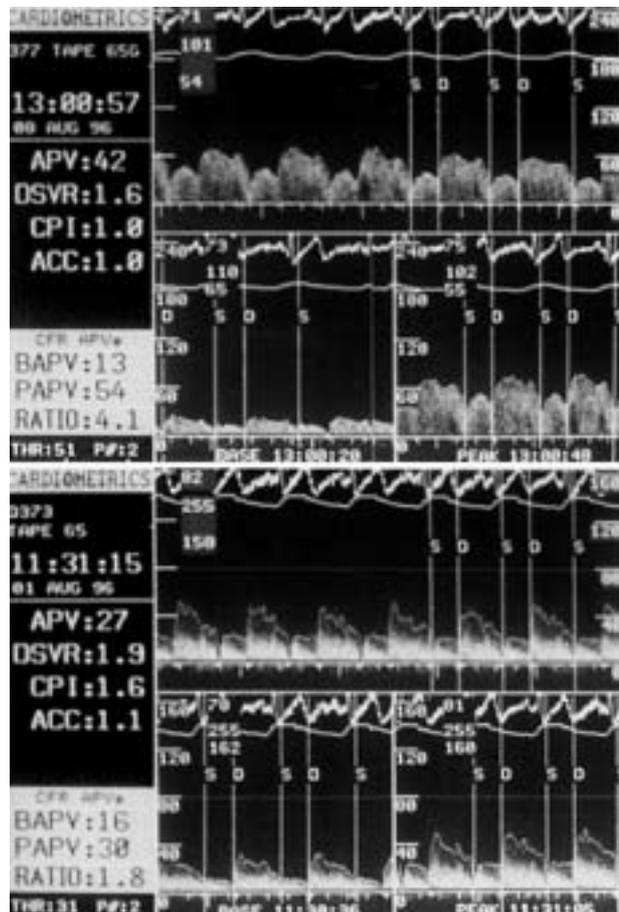

Figure 1. Intracoronary Doppler display representing intracoronary flow velocity in response to intracoronary adenosine for the evaluation of coronary flow reserve, which is calculated as the ratio of the peak velocities at maximal hyperemia divided by the baseline velocities. Top image: normal response to intracoronary adenosine with a coronary flow reserve of 4.1. Bottom image: abnormal coronary flow reserve of 1.8.

with two-dimensional imaging, color and spectral Doppler, internal electrocardiographic monitoring, and high frequency vascular tranducer are needed for the measurement. After a baseline resting image and blood flow have been recorded, a blood pressure cuff is placed either above the antecubital fossa or on the forearm and inflated to at least 50 mm above the systolic blood pressure, to occlude the blood vessel. The occlusion causes vasodilatation of the downstream resistance vessel. After deflation of the cuff, there is a transient high flow state (reactive hyperemia). At the brachial artery level, the endothe-

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lium responds to an increase in blood flow, as sensed by increased shear stress, by releasing nitric oxide, and subsequently results in vasodilatation. This endothelium-dependent phenomenon is known as flow-mediated vasodilatation. An ultrasound image of the brachial artery is recorded from 30 seconds before to 2 minutes after deflation of the cuff. The flow-mediated vasodilatation is reported as either absolute change or percentage change in diameter. Despite its utility, this method has technical and interpretive limitations. For details, refer to the American College of Cardiology guidelines [14].

The prognostic indications of forearm endothelial function quickly expanded. Neunteufl et al [7] showed that a reduced brachial artery flow-mediated vasodilatation response to reactive hyperemia (less than 10% increase) in patients with angina is correlated with increased death, myocardial infarction, or a need for revascularization. Heitzer et al [8] showed that a reduced brachial artery blood flow response to adrenocorticotrophic hormone in patients with coronary artery disease diagnosed by angiogram was associated with increased death, myocardial infarction, need for revascularization, and ischemic stroke. Thus, assessment of forearm endothelial dysfunction may allow for expansion of the utilization of endothelial dysfunction.

Clinical applications of measurement of endothelial dysfunction

Clinical application in hypertension

In patients with hypertension, endothelial dysfunction is a predictor of adverse outcome. Perticone et al [9] conducted a 31.5-month follow-up study in 225 never-treated patients with hypertension. Patients were stratified into three groups on the basis of their percentage increase in forearm blood flow from basal: group 1, 30–184%; group 2, 185–333%; group 3, 339–760%. In group 1, the relative risk for cardiovascular events was 2.084 times that of group 3 ($P = 0.0049$). In multivariate analysis, the only independent predictors of cardiovascular events were mean 24-hour ambulatory blood pressure and the peak percent increase in forearm blood flow. Thus, measuring endothelial function may allow clinicians to identify a subgroup of patients at greatest risk, in whom aggressive treatment is warranted.

Endothelial dysfunction may be used to identify normotensive people who have a genetic predisposition to cardiovascular disease. Normotensive individuals with a family history of hypertension have a significantly depressed forearm endothelial function index, calculated as the ratio between endothelium-dependent and endothelium-independent vasodilatation. Likewise, healthy individuals reporting at least one parent suffering from myocardial infarction showed a significantly lower EDV than individuals without such a family history. A prospective follow-up study will be necessary to determine whether endothelial dysfunction in normal individuals is indeed predictive of future hypertension and myocardial infarction [15].

Clinical application in congestive heart failure

Congestive heart failure is also related to endothelial dysfunction. The relationship of disease severity to the level of endothelial dysfunction was investigated by Bank et al [16], who found that endothelial dysfunction measured by the forearm blood flow response to methacholine was present and near maximum in mild congestive heart failure. Thus endothelial dysfunction per se, rather than disease severity index, may serve as an early disease marker in congestive heart failure. Another finding by Bank et al [16] was that both endothelium-dependent and endothelium-independent vasodilatation were impaired in congestive heart failure. The same finding was reported by Maguire et al [17] and Negrao et al [18], suggesting that both endothelium and smooth muscle are dysfunctional in congestive heart failure.

Clinical application in hypercholesterolemia and obesity

Data from the early 1990s had shown that endothelium-dependent vasodilatation is impaired in patients with hypercholesterolemia [19–21]. More recent studies have shown that hypertriglyceridemia further impairs endothelial function in patients with and without hypercholesterolemia [22,23]. In contrast, an increased high-density lipoprotein concentration in patients with hypercholesterolemia improves en-

dothelial function [24]. Obesity also adversely affects endothelial function in normotensive and hypertensive patients, suggesting that obesity is an independent risk factor for endothelial dysfunction [25].

Clinical application in noncardiologic conditions such as portal hypertension and cirrhosis

The concept of endothelial dysfunction has been a focus of clinical research not only in cardiology but also in noncardiologic conditions. For example, cirrhosis of the liver is often accompanied by the morbid complication of portal hypertension. A component of portal hypertension occurs through endothelial dysfunction and subsequent vasoconstriction within the hepatic sinusoids, and this may represent a target for treatment of portal hypertension in humans through approaches that aim to supplement the generation of nitric oxide in the liver [26].

Marker for therapeutic response and implication for future studies

Improvement in endothelial function has been used as a surrogate marker of therapeutic response. Studies have shown that it can be achieved through exercise in patients with coronary artery disease [27], congestive heart failure [28], and type 2 diabetes mellitus [29], although the effect obtained through exercise in healthy individuals remains controversial [30]. Conversely, smoking [31] and high-fat meals [32] adversely affect endothelial function. In common with measurement of weight, blood pressure, and cholesterol, measurement of endothelial function can perhaps be used as a feedback to encourage and monitor therapeutic lifestyle change.

Analysis of endothelial function is now a frequent end point of research studies. A study that measures endothelial dysfunction, a surrogate marker of cardiovascular events, does not have as much power as a study that uses cardiovascular events as an end point. However, a study that measures endothelial dysfunction can be reasonably accomplished in a few months, whereas one that uses cardiovascular events as an end point will usually take years. Thus analysis of endothelial dysfunction has both clinical and research utility.

Conclusion

Studies have shown that endothelial dysfunction may be a predictor of cardiovascular events. It can be measured in the coronary artery and brachial artery, and the results are closely correlated with each other. The relevant study population includes those with coronary artery disease, hyperlipidemia, hypertension, and obesity, and healthy individuals. Future expansion of the practise of clinical measurement of endothelial dysfunction in humans will be determined by continued studies aimed at establishing its clinical utility, and by continued technical advances aimed at improving its ease of use and applicability. ■

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Imaging of coronary endothelial dysfunction by use of positron emission tomography

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Abstract

Endothelial dysfunction is recognized as a pivotal event early in the development of coronary atherosclerosis, and techniques for its detection may be of significant value. At present, positron emission tomography (PET) is the only imaging method that provides noninvasive, quantitative information about vascular reactivity and endothelial function at the level of the myocardial microcirculation. It has been used with success in the description of the effects on myocardial blood flow of hyperlipidemia, smoking, diabetes, and other risk factors. It holds promise for the identification and selection of individuals at greatest risk for progression to clinical coronary artery disease. In the light of the increasing numbers of therapeutic agents targeting endothelial function, the importance of imaging as a surrogate marker of efficacy will also increase.
■ *Heart Metab.* 2004;22:17–21.

Keywords: Endothelial dysfunction, coronary microcirculation, nuclear cardiology, positron emission tomography, myocardial blood flow, cold pressor test

Introduction

Nowadays the importance of endothelial integrity for the regulation and maintenance of normal vascular function is fully recognized. Clinically, a paradigm change in coronary artery disease has occurred. In past trials of lifestyle modification and lipid lowering, significant reductions in cardiac event rates were noted, despite there being little or no effect on the degree of coronary stenoses. The emphasis in the prevention of disease progression has thus shifted from modification of structural changes towards modification of functional alterations [1]. In addition, advances in basic science have contributed substantially to an increase and refinement of understanding of the role of endothelial dysfunction in early development and progression of atherosclerosis [2]. It has been demonstrated that individuals with impaired endothelial function are at increased risk for future development of overt coronary heart disease and for occurrence of cardiac events [3,4]. Reliable biologic imaging techniques that allow for testing of endothelial integrity are therefore sought, and could be of considerable clinical value.

Comparison of techniques for identification of endothelial dysfunction

Endothelial function can be tested at several vascular sites (*Table I*). Increases in forearm blood flow or brachial artery diameter in response to endothelium-specific stimuli such as transient occlusion are assessed by ultrasound and used as an indicator of the integrity of the endothelium. These measurements in peripheral vessels are then extrapolated to the coronary circulation. Questions remain, however, as to whether functional alterations in peripheral vessels always reflect alterations in the coronary circulation. Recently, for example, it was reported that there was no correlation between peripheral perfusion responses to transient forearm ischemia and dipyridamole-induced myocardial hyperemia, in groups of

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Table I. Methods for detection of endothelial dysfunction.

Imaging method	Target structure	Technique	Drawbacks
Ultrasound	Peripheral vessels	Artery diameter, Doppler flow	Extrapolation to coronary vessels
Catheterization	Large coronary arteries	Doppler flow wire, artery diameter	Invasiveness
Positron emission tomography	Coronary microcirculation	Quantification of tissue flow	Limited availability

healthy normal individuals, patients with coronary artery disease, and patients with syndrome X, suggesting that extrapolation of findings between the two vascular beds is not feasible [5].

Invasive measurements of Doppler flow velocity or angiographic vessel diameters allow for testing of endothelial function of epicardial conduit vessels. Intracoronary injection of adenosine or papaverine normally results in a flow mediated (or shear stress mediated) increase in the release of nitric oxide and an increase in vessel diameter. A similar effect is provoked by intracoronary acetylcholine, which stimulates the release of nitric oxide directly via muscarinic endothelial receptors. The invasiveness of these approaches, however, precludes their large-scale and repetitive clinical use.

By providing qualitative and, in particular, quantitative information about myocardial blood flow at baseline and in response to various stimuli, positron emission tomography (PET) is at present the only imaging modality that makes possible the noninvasive assessment of endothelial function at the level of the coronary microvasculature.

Quantification of myocardial blood flow by positron emission tomography

Regional myocardial blood flow is generally measured using two approaches, one based on the freely perfusible tracer, [^{15}O]-water, and the other based on the metabolically trapped perfusion tracer, [^{13}N]ammonia. Dynamic imaging, creation of time–activity curves for blood and myocardium, and curve fitting to compartment models are necessary steps for quantification. Both approaches have been extensively validated against independent microsphere blood flow measurements in animals and their reproduci-

bility has been demonstrated, so that they are readily available for investigational and routine clinical use [6]. Current methodology allows for three-dimensional volumetric assessment of global and regional myocardial flow at rest and in response to stress stimuli (*Figure 1*). These parameters reflect tissue perfusion and thus the integrated function of the coronary circulation of the heart at the resistance level.

Endothelium-specific stress testing for positron emission tomography imaging

Typically, vasodilators such as adenosine or dipyridamole have been used in stress imaging in PET studies [6]. Those agents act directly on vascular smooth muscle cells via specific adenosine receptors, causing relaxation and thus increased vasodilatation and flow. Hyperemic flow in response to these agents normally increases 2.5- to 5-fold from baseline, and is believed to reflect an integrated response of the coronary circulatory system, partially mediated by direct smooth muscle effects and partially mediated by additional endothelial activation related to shear stress (*Figure 2a*) [7].

More recently, stress tests that are specific for endothelial function have been applied in positron emission tomography imaging. These are based on sympathetic stimulation, either by cold pressor test [8,9] or by mental stress [10]. Release of norepinephrine from stimulated sympathetic neurons activates α-adrenoceptors on the endothelium that mediate the release of nitric oxide (*Figure 2b*). This vasodilatory signal results in a 30–50% increase in baseline flow in the presence of an intact endothelium. α-Adrenergic stimulation of vascular smooth muscle cells, which causes vasoconstriction, is
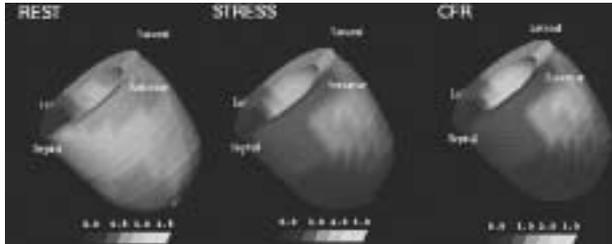
Figure 1. Three-dimensional parametric polar maps of left ventricular myocardial blood flow determined by [^{13}N]ammonia positron emission tomography at rest (left) and during pharmacologic hyperemia (middle). Right: myocardial flow reserve.
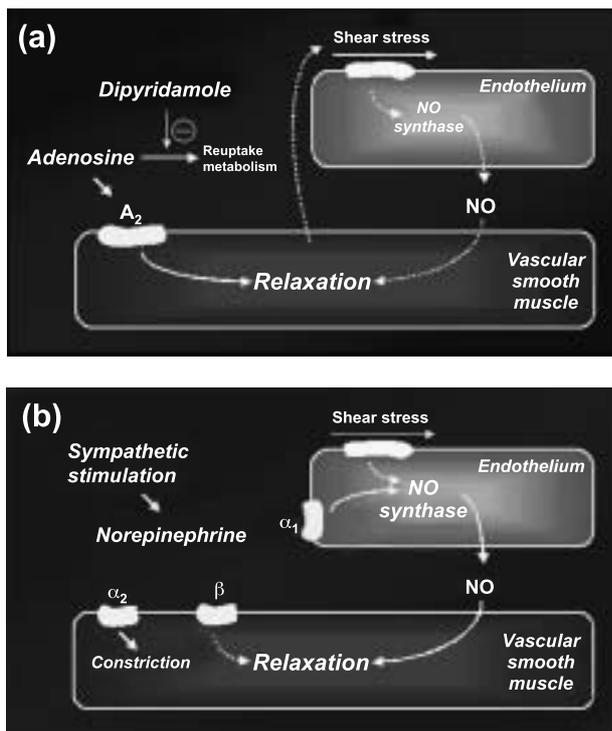

Figure 2. Schematic representation of endothelium-dependent and endothelium-independent effects of (a) pharmacologic vasodilatation and (b) sympathetic stimulation. A_2, adenosine A_2-receptor; α_1, α_2, β, α_1-, α_2-, and β-adrenergic receptors; NO, nitric oxide.

normally counteracted, but may outweigh the endothelium-derived vasodilatation in the presence of impaired endothelial integrity. The resulting decrease in flow then indicates endothelial dysfunc-

tion. PET flow measurements during sympathetic stimulation are therefore believed to provide specific information about coronary endothelial function.

Characterization of the effects of traditional risk factors

Several early studies using PET at rest and during pharmacologic hyperemia demonstrated impairments in flow reserve in groups of patients with risk factors but no clinical evidence of coronary disease. A relationship with age was observed, characterized by a reduction in flow reserve in older individuals as a result of an increase in resting flow and a less pronounced decrease in hyperemic flow [11,12]. Further studies established positive correlations between impairment of lipid profile and microvascular reactivity [13,14]. Decreased flow reserve in association with increasing total and low-density lipoprotein cholesterol concentrations has been demonstrated in dyslipidemic individuals, whereas high-density lipoprotein cholesterol seems to be associated with greater vascular reactivity in healthy individuals [15].

Initial studies indicated an impaired flow response to dipyridamole during acute smoking in new smokers, but no impairments in chronic smokers [16]. More recent studies in this group of individuals at risk have involved the application of endothelial-dependent cold pressor testing to gain further insight into vascular reactivity. Again, chronic smokers exhibited no impairment of hyperemic flow in response to pharmacologic vasodilatation, but their response to sympathetic stimulation by cold pressor testing was significantly impaired compared with that of normal individuals [8]. Furthermore, this impaired response to cold was alleviated by application of L-arginine, a precursor of nitric oxide and substrate of nitric oxide synthase [17].

Diabetes mellitus is another cardiovascular risk factor that has been evaluated extensively. Impairments of flow reserve in response to pharmacologic vasodilatation have been demonstrated in insulin-dependent and non-insulin-dependent diabetic individuals early in the course of their disease [18–20]. More recently, an impaired response to cold has been identified in one third of a group of asymptomatic patients with mild diabetes controlled by diet [21]. Effects of impaired glucose tolerance, insulin, and the

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metabolic syndrome on microvascular reactivity are the subject of continuing and recently published studies [22].

Assessment of preventive and therapeutic interventions

On the basis of observations in groups of patients with specific risk factors, several strategies for prevention, risk factor modification, and medical treatment have been evaluated in coronary artery disease, with regard to their effects on myocardial blood flow and vascular reactivity. Using PET, improvements in perfusion have been observed as a consequence of short-term cardiovascular conditioning, low-fat diet, and long-term modification in risk factors [23–25]. Beneficial effects of antioxidant vitamins have been observed, especially in smokers [26].

In addition, the effects of specific drugs have been evaluated. Antihypertensive agents and lipid-lowering drugs were shown to improve flow reserve in individuals with mild coronary disease, substantiating their potential in secondary prevention [27,28]. More recently, effects of estrogens have been evaluated, yielding no improvement in vascular response to hyperemia or to cold in women with risk factors, and only mild improvement in endothelium-dependent response to cold in otherwise healthy women [29,30]. The potential of PET to serve as a surrogate marker of drug effectiveness has been increasingly emphasized, and the number and size of clinical trials using PET flow measurements for the evaluation of the effects of drugs is increasing. The greater demand for this imaging procedure will not only increase its recognition, but may also stimulate its evaluation as a feasible diagnostic and prognostic tool in a clinical setting in the future.

Summary and conclusion

Positron emission tomography makes possible non-invasive, quantitative assessment of coronary microvascular reactivity and endothelial function. Studies in individuals with cardiovascular risk factors have demonstrated that abnormalities are present before the development of structural vascular changes. Further studies have also demonstrated that such abnormalities can be reversed by specific therapeutic interventions. Determination of whether such improvements in vasomotion translate into long-term benefits and improved outcome will be important. Nevertheless, imaging of endothelial function will have an increasing role as a surrogate marker of efficacy in clinical trials of preventive and novel pharmacotherapeutic strategies for cardiovascular disease. By targeting the earliest functional alterations that precede morphologic atherosclerotic changes, PET also has the potential to emerge as a future clinical tool for use in individuals at high cardiovascular risk. ■

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Treatment options for endothelial dysfunction

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Abstract

Endothelial dysfunction is frequently observed in different cardiovascular diseases. Currently, there is no specific treatment for this perturbation, although different therapeutic approaches have been proposed. Angiotensin-converting enzyme inhibitors are well established in the treatment of different cardiovascular illnesses, and they are known to improve endothelial function. Recent studies have demonstrated that statins also have the potential to ameliorate endothelial dysfunction. The aim of this review is to discuss possible therapeutic approaches to endothelial dysfunction, focusing particularly on the mechanisms of action of both angiotensin-converting enzyme inhibitors and statins.
■ *Heart Metab.* 2004;22:22–28.

Keywords: Endothelial function, nitric oxide, angiotensin-converting enzyme inhibitor, statin, therapy

Introduction

Far from being inert, the vascular endothelium is an important source of mediators, which act predominantly in a paracrine fashion. These mediators maintain an antithrombotic surface, regulate vascular tone, modulate inflammatory responses, and inhibit proliferation of vascular smooth muscle cells [1]. The most important such mediator is nitric oxide, which is constitutively produced by endothelial nitric oxide synthase (eNOS). In addition, the endothelium expresses angiotensin-converting enzyme, which converts angiotensin I into the potent vasoconstrictor, angiotensin II. Normally, the production of vasoactive substances favors vasodilation; endothelial dysfunction has, therefore, widespread consequences. The condition is seen in different chronic illnesses, such as hypercholesterolemia, atherosclerosis, hypertension, chronic heart failure, and certain inflammatory diseases. Indeed, endothelial dysfunction appears to be a useful marker of early stages of various cardiovascular illnesses [2]. However, this perturbation has also been reported in normotensive individuals who merely have a family history of cardiovascular risk factors [3]. It has therefore been suggested that the onset of endothelial dysfunction may precede the development of clinically evident vascular disease in many cases [4].

Several factors contribute to a lack of nitric oxide in endothelial dysfunction. Importantly, the increased production of reactive oxygen species, such as superoxide anion, enhances nitric oxide breakdown. Achieving an increase in the production of nitric oxide and/or reducing the amount of reactive oxygen species in the endothelium appears to be a promising approach to treat endothelial dysfunction. However, other features may also be important. The expected result from such treatment would be a decrease in the number of clinical events. This review will focus on 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) and angiotensin-converting enzyme (ACE) inhibitors. Several other substances have also proven beneficial in diseases that are accompanied by endothelial dysfunction.

Statins

Statins were originally designed to decrease plasma cholesterol concentrations. Five different statins are currently available, and the development of new substances is well under way. Strong and consistent evidence suggests that decreasing plasma low-

density lipoprotein (LDL) concentrations alone by means of diet and plasma apheresis improves endothelial function [5,6]. Statin treatment consistently reduces cardiovascular risk [7,8] and reverses endothelial dysfunction [9,10]. Indeed, a reduction in recurrent coronary events has been observed as early as 16 weeks after the initiation of treatment [11], and it appears that these beneficial effects are independent of cholesterol decreasing activity [12].

These so-called pleiotropic effects of statins have been the subject of considerable research over recent years. Some effects are attributable to the inhibition of cholesterol biosynthesis, because substrates downstream from mevalonate in the synthesis cascade supply a number of different metabolic pathways (*Figure 1*) [13]. One such substrate is geranylgeranyl-pyrophosphate, which serves as a
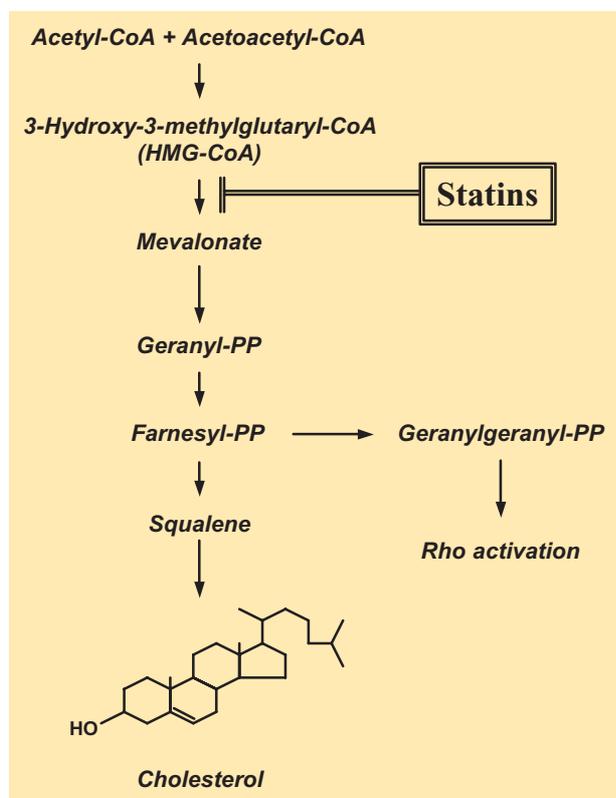

Figure 1. Pathway of cholesterol biosynthesis. The rate-limiting step is 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase activity. This enzyme is competitively inhibited by statins. Intermediates are used as attachments to different proteins and enzymes. CoA, coenzyme A; PP, pyro-phosphate.

lipid attachment to Rho (*Figure 2*). This guanosine triphosphate-binding protein coordinates a number of specific cellular responses by interacting with downstream targets [14], and it is involved in stress fiber formation [15], monocyte adhesion, and monocyte transmigration through the endothelium [16,17]. Other mechanisms of statin action are less well understood, although these effects are likely to improve endothelial function via direct and indirect mechanisms (*Figure 2*). Lovastatin and simvastatin, for example, have been shown to induce *eNOS* gene transcription in human endothelial cells [18]. Interestingly, pravastatin improved endothelial function in monkeys at doses that do not decrease plasma LDL concentrations [19]. In this study, 32 cynomolgus monkeys were fed an atherogenic diet for 2 years, followed by a 2-year treatment phase in which they were fed a lipid-decreasing diet containing (n = 14) or not containing (n = 18) pravastatin. Coronary arteries of those monkeys treated with pravastatin dilated ($10 \pm 3\%$), whereas those of control monkeys constricted ($-2 \pm 2\%$, $P < 0.05$), in response to acetylcholine. Pravastatin has also been shown to increase the bioavailability of nitric oxide in atherosclerotic arterial walls [19], and it activates eNOS independently of its cholesterol-decreasing features [20].

Some antioxidant properties of statins have recently been documented. The two most likely sources of superoxide anion are mitochondria and immune cells, although the formation of uric acid also yields this reactive oxygen species (*Figure 2*) [21]. A diversity of antioxidant systems, such as superoxide dismutase and catalase, counteract the continuous generation of reactive oxygen species. Atorvastatin has recently been shown to upregulate the expression of catalase at the mRNA and protein levels in cultured rat aortic vascular smooth muscle cells [22]. However, the activity of superoxide dismutase was unaffected [22]. In this study, both angiotensin II-induced and epidermal growth factor-induced production of reactive oxygen species were down-regulated [22]. These mechanisms may therefore contribute to the vasoprotective effects of statins. However, the pathways involved may still be indirect [23]. Statins also appear to be involved in an enhancement of neovascularization. Indeed, simvastatin has been shown to augment the circulating pool of bone marrow-derived endothelial progenitor cells [24].

New therapeutic approaches
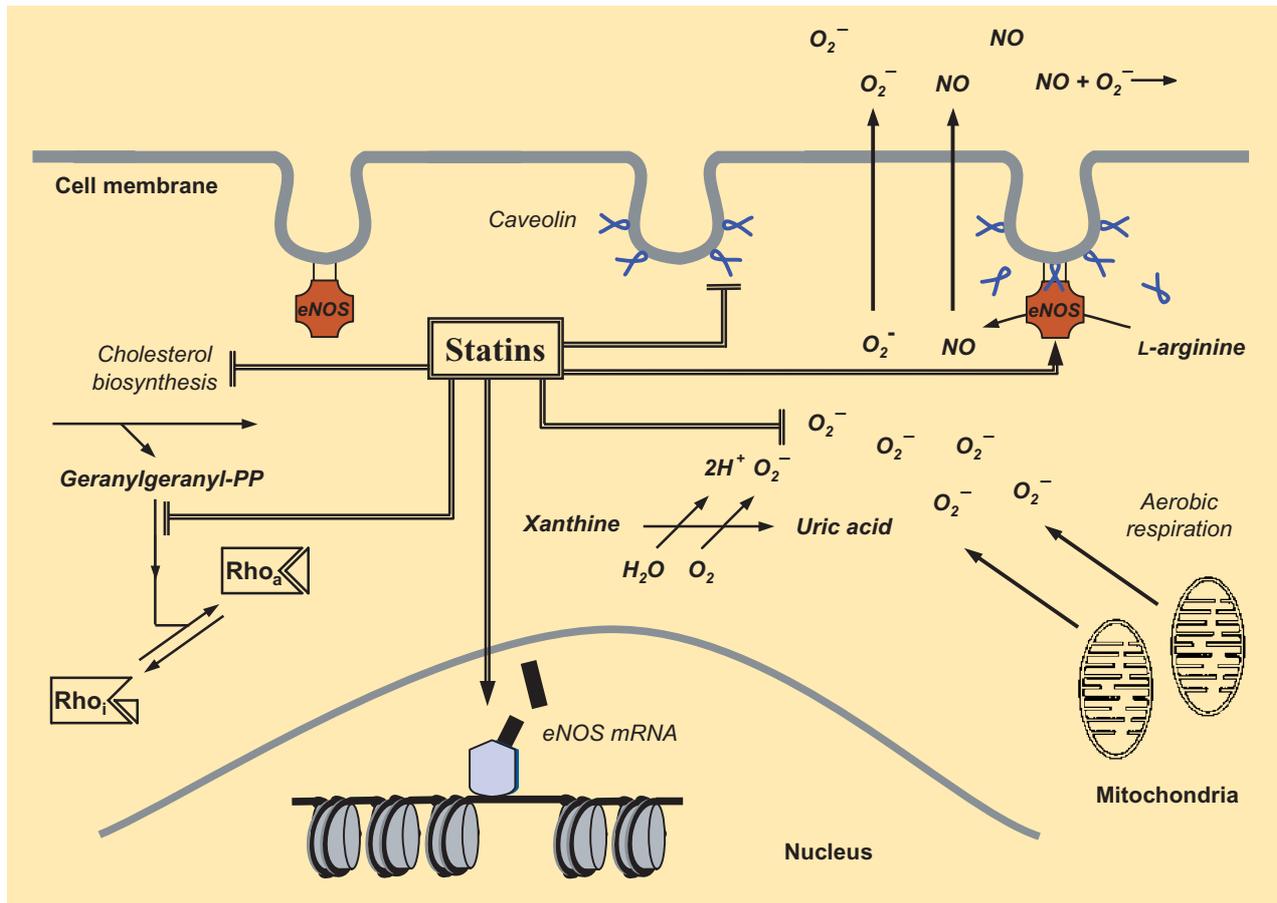
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Figure 2. Statin-mediated effects in endothelial cells and other tissues. Statins inhibit the production of geranylgeranyl-pyrophosphate (PP) through blocking cholesterol biosynthesis, which leads to an impairment of Rho activation. Inactive Rho (Rho$_i$) accumulates in the cytosol. Statins also induce endothelial nitric oxide synthase (eNOS) gene transcription and augment eNOS protein activity. Moreover, statins prevent expression of caveolin, a caveolae-associated protein that inhibits eNOS activity. Finally, statins also inhibit oxidative stress, although the mechanism may be indirect. eNOS, endothelial nitric oxide synthase; NO, nitric oxide; O_2^-, superoxide anion; $ONOO^-$, peroxynitrite; Rho$_a$, active Rho.

Statin-mediated effects on the abundance of caveolin also appear to be involved in improving endothelial function. Caveolin is a marker protein of specific cell membrane invaginations (caveolae), which display the greatest cellular eNOS activity (*Figure 2*). Caveolin appears to interact directly with eNOS [25], and it inhibits the production of nitric oxide. Two recent studies have shown that both atorvastatin and the new substance, rosuvastatin, decrease the expression of caveolin, which ultimately leads to an increased production of nitric oxide [26,27]. Indeed, treatment with rosuvastatin for 2 weeks decreased the expression of aortic caveolin protein in apolipoprotein E-deficient mice by 2.0-fold as compared with control mice [27].

Most recently, statin-mediated anti-inflammatory effects have been observed. Simvastatin pretreatment, for example, inhibited *Staphylococcus aureus*-induced leukocyte rolling and adherence, as assessed by intravital microscopy in the rat mesenteric circulation [28]. Leukocyte transmigration was also significantly decreased by such treatment [28]. Another study found that pravastatin decreases the concentrations of the acute-phase reactant C-reactive protein after myocardial infarction and in patients with hypercholesterolemia [29]. As the proinflammatory cytokine, tumor necrosis factor-α, is known to
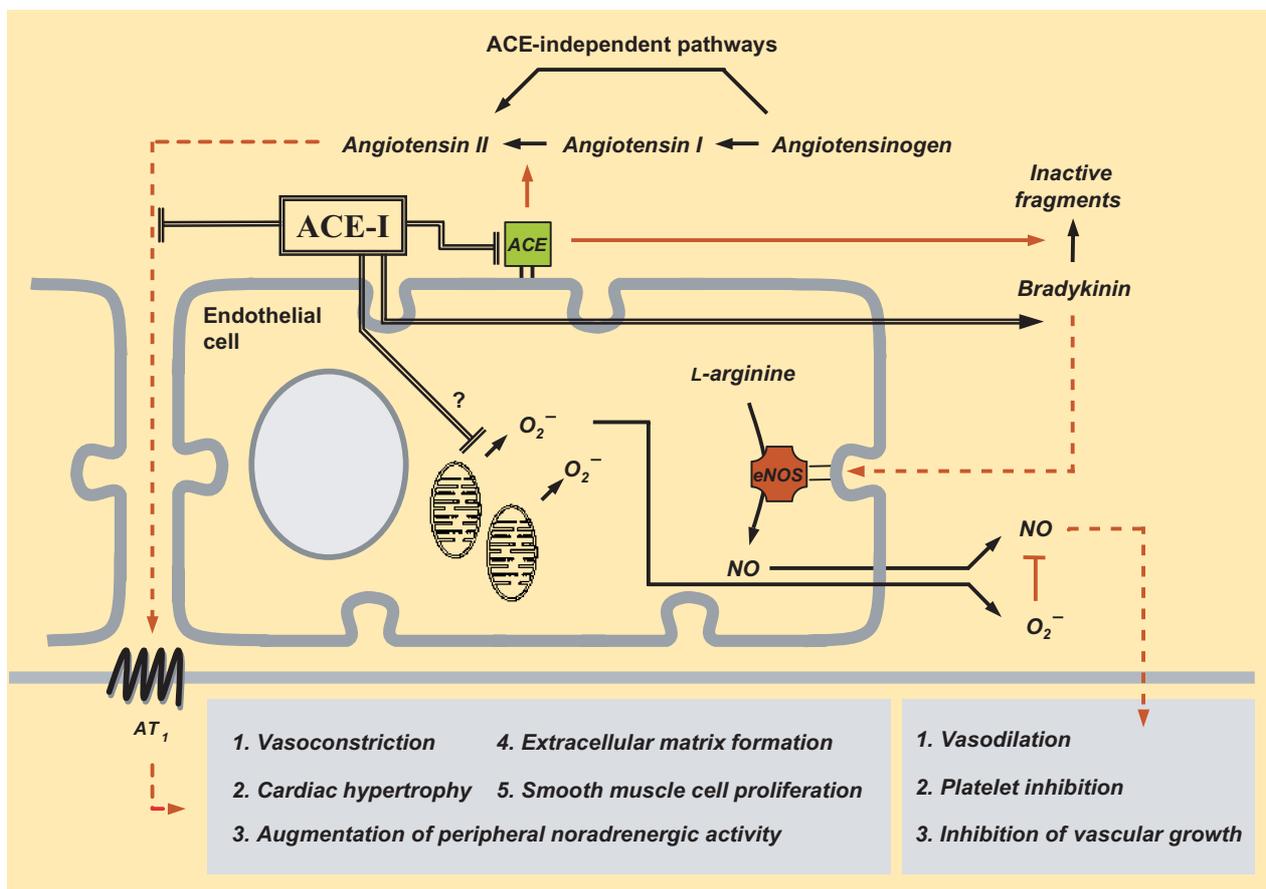
Figure 3. Angiotensin-converting enzyme inhibitor (ACE-I)-mediated effects on endothelial function. ACEIs block the conversion of angiotensin I to angiotensin II, although some angiotensin-converting enzyme (ACE)-independent pathways still supply a small amount of the latter peptide. As ACE also degrades bradykinin, ACE inhibitors stop the breakdown of this substance, which eventually increases the activity of endothelial nitric oxide synthase (eNOS). ACE inhibitors may also interfere with the production of reactive oxygen species, such as superoxide anion (O_2^-), although the mechanism involved appears to be indirect. AT_1, angiotensin II type 1 receptor; NO, nitric oxide.

worsen endothelial dysfunction, it is interesting to note that lovastatin has been demonstrated to inhibit the induction of this and other proinflammatory substances in macrophages [30]. The stimulus for production of tumor necrosis factor-α remains a matter of debate, but it seems that bacterial lipopolysaccharide has a significant role [31,32].

Angiotensin-converting enzyme inhibitors

Angiotensins are peptides derived from their precursor, angiotensinogen. The classic pathway of angiotensin synthesis includes a reaction catalyzed by ACE, although angiotensin II, the principal effector of the system, can also be synthesized independently of this enzyme (*Figure 3*) [33]. Most actions of angiotensin II support or increase arterial blood pressure and maintain glomerular filtration. Vasoconstriction, mediated by this peptide, occurs within seconds [33]. Other actions of angiotensin II, such as vascular growth and ventricular hypertrophy, take days or weeks to occur [34].

In addition to their established efficacy in decreasing blood pressure, ACE inhibitors have the broadest impact of any drug in cardiovascular medicine, reducing the risk of death, myocardial infarction, stroke, diabetes mellitus, and renal impairment [35].

New therapeutic approaches
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In large outcome trials, ACE inhibition has been documented to reduce cardiovascular events in patients with coronary artery disease, heart failure, and related cardiovascular pathologies [36–40]. Similarly, ACE inhibitors are known to improve endothelial function. This was shown, for example, in a prospective, randomized, parallel group study [41]. Endothelial function was assessed in a population of 168 patients with hypertension, before and after 6 months of treatment. Patients were randomly assigned to receive nifedipine (n = 28), amlodipine (n = 28), atenolol (n = 29, nebivolol (n = 28), telmisartan (n = 29), or perindopril (n = 28). All treatments reduced blood pressure to a similar extent as compared with healthy control individuals (n = 40), but flow-mediated dilatation was increased only in the perindopril group (to $6.4 \pm 2.4\%$) as compared with baseline ($5.1 \pm 2\%$ to $6.4 \pm 2.4\%$) and the other treatment regimens ($1.5 \pm 2.1\%$; $P < 0.01$), without modifying the response to glyceryl trinitrate. After perindopril treatment, the endothelium-dependent vasodilatation in patients with hypertension was no longer different from flow-mediated dilatation in normotensive individuals.

Another double-blind, randomized, placebo-controlled study compared the effect of quinapril 40 mg once daily with that of placebo, in 105 normotensive patients with coronary artery disease [42]. Using quantitative angiography, it could be demonstrated that the quinapril group showed a significant improvement in coronary artery diameter in response to incremental concentrations of acetylcholine (quinapril compared with placebo: $4.5 \pm 3\%$ compared with $-0.1 \pm 3\%$ at 10^{-6} mol/L; $12 \pm 3\%$ compared with $-1 \pm 3\%$ at 10^{-4} mol/L; $P = 0.002$) [42].

Several mechanisms may contribute to the effect of ACE inhibitors and angiotensin receptor blockade on endothelial function. Indeed, angiotensin II increases the production of reactive oxygen species and hence the inactivation of nitric oxide [43]. The reason for the increase in oxidative stress is the induction of nicotinamide adenine dinucleotide phosphate oxidase activity [44]. The generation of reactive oxygen species also has a crucial role in promoting atherosclerosis by different mechanisms, such as oxidation of LDL cholesterol and upregulation of leukocyte adhesion molecules [4,45]. Besides reducing oxidative stress, ACE inhibition leads to a decrease in bradykinin breakdown, which in turn stimulates the production of nitric oxide (*Figure 3*) [43]. The balance between angiotensin II and nitric oxide has been suggested as a major determinant of endothelial and vascular phenotype [4].

Conclusions

Endothelial dysfunction has been recognized as a major clinical syndrome accompanying and worsening many cardiovascular diseases. Several drugs have been shown to improve endothelial function in such conditions, although this effect is currently only a ''side effect'' of treating the underlying disorder. It is tempting to speculate that the endothelium may be a direct target for future therapeutic interventions. ACE inhibitors have already been shown to improve endothelial function in patients with cardiovascular diseases [41,42]. Statins may also prove effective in this setting [46], although their potential to decrease plasma cholesterol concentrations is not always wanted. Indeed, patients with chronic heart failure suffer from endothelial dysfunction, but low plasma LDL concentrations appear to correlate with poor outcome [47]. The reason for this may lie in the fact that cholesterol potentially inactivates the activity of bacterial lipopolysaccharide in the circulation, which normally triggers the production of tumor necrosis factor-α [47]. Therefore, large-scale trials are needed to establish the right doses in the right patients. This implies that very low doses of statins *could* still yield their beneficial pleiotropic effects without decreasing plasma cholesterol.

Summary

Endothelial dysfunction plays a significant part in various cardiovascular diseases. Therapeutic approaches to treat this perturbation have so far mainly dealt with the underlying disorder, and treatment of the endothelium was merely a ''side effect.'' This is true for ACE inhibitors, which have proven beneficial in this setting. Statins are of particular interest, because these substances counterbalance different parts of this condition. Future therapies will target the endothelium directly, in the hope that this will yield a reduction in clinical events.∎

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Evidence-based efficacy of Vastarel in patients with ischemic cardiomyopathy

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Abstract

During myocardial ischemia, significant metabolic changes occur at the cellular level (such as intracellular acidosis, reduction in high energy substrate, and onset of anaerobic metabolism), which in turn trigger intracellular alterations leading to contractile dysfunction, electrocardiographic changes, and anginal pain. Metabolically active drugs serve as important alternatives to conventional antianginal therapy that modify myocardial oxygen supply and demand through alterations in coronary blood flow, blood pressure, and heart rate. By switching the substrate energy preference for cellular metabolism, Vastarel MR (trimetazidine) proved to be effective in treating patients with angina pectoris. By the same effect, it can also improve left ventricular function in patients with chronic ischemic cardiomyopathy or diabetic cardiomyopathy and by limiting infarct size after reperfusion therapy in patients who have suffered myocardial infarction.
■ *Heart Metab.* 2004;22:29–32.

Keywords: Myocardial ischemia, cardiomyopathy, trimetazidine

Introduction

The use of metabolic agents in patients with overt myocardial ischemia in the form of stable angina pectoris is well recognized. Through selective inhibition of 3-ketoacyl coenzyme A thiolase, an enzyme of fatty acid β-oxidation, Vastarel MR increases the oxidation of pyruvate formed from glucose, glycogen, and lactate, and decreases the use of free fatty acids as a myocardial fuel source. The resulting enhanced cardiac efficiency and energy production increase the myocardial cellular ischemic threshold and provide cytoprotection. These beneficial effects occur in the absence of significant changes in hemodynamic parameters, including heart rate and systolic blood pressure. Clinical studies have established the efficacy of Vastarel MR in improving exercise duration time, time to angina, and exercise capacity in patients with stable angina pectoris. By the same cytoprotective effect, Vastarel MR can also improve the left ventricular dysfunction of patients with coronary artery disease. This paper reviews the literature on the benefits of Vastarel in patients with ischemic cardiomyopathy. *Table I* summarizes the findings of some relevant studies.

Effect of Vastarel in chronic ischemic left ventricular dysfunction

Brottier et al [1] were the first to report the effect of Vastarel on severe ischemic cardiomyopathy. Their group of patients with ischemic congestive cardiomyopathy and severely depressed ventricular ejection fraction were given oral Vastarel for 6 months. By 6 months, radionucleide ejection fraction had increased by more than 9%, in relative terms, and functional capacity had improved. These improvements occurred in the absence of obvious hemodynamic changes and the effect was probably attributable to improved myocardial metabolism.

Further support for the role of Vastarel in patients with moderate chronic ischemic dysfunction was provided by Lu et al [2], who showed that this agent not only prevents and delays the progression of ischemic cardiomyopathy, but also improves the resting left ventricular function of patients. In a double-blind, placebo-controlled, crossover design study in 15 patients with documented chronic coronary artery disease, the patients were randomly assigned to receive Vastarel or placebo in addition to

Focus on Vastarel MR

Huay Cheem Tan

Table I. Effects of trimetazidine in ischemic cardiomyopathy.

Study	No. patients	Effect of trimetazidine
Brottier [1]	20	Improved LVEF
		Improved CHF symptoms
Lu et al [2]	15	Improved LVEF
		Reduced dobutamine-induced ischemia
Belardinelli and Purcaro [3]	38	Improved LVEF
		Improved left ventricular wall motion score index
Fragasso et al [5]	13	Improved LVEF
		Improved left ventricular fractional shortening

CHF, congestive heart failure; LVEF, left ventricular ejection fraction.

their usual antianginal medications. Crossover took place after 15 days and the duration of the trial was 30 days. All patients underwent dobutamine echocardiography at day 0, day 15, and day 30. The mean period of dobutamine infusion required to bring about the onset of new dysfunction, or worsening of pre-existing dysfunction, was 15.2 ± 4.1 min with placebo and increased to 17.5 ± 4.9 min with Vastarel ($P = 0.04$). The mean dose of dobutamine required to bring about the changes was 22.1 ± 5.8 μg/kg per min with placebo and increased to 27.9 ± 8.0 μg/kg per min with Vastarel ($P = 0.006$). Both in the resting condition and at peak dobutamine infusion, wall motion score index was significantly lower with Vastarel than with placebo (at rest: 1.34 ± 0.37 compared with 1.40 ± 0.42, $P = 0.013$; at peak: 1.61 ± 0.40 compared with 1.71 ± 0.45, $P = 0.018$). These results were achieved with no effect on the patients' heart rate, systolic blood pressure, and rate–pressure product. They indicate that Vastarel not only may protect the heart from dobutamine-induced ischemic dysfunction, but also can improve resting regional left ventricular function as demonstrated by improved peak and resting wall motion score index.

Similar improvement in patients with severe ischemic cardiomyopathy was observed by Belardinelli and Purcaro [3]. They studied 38 patients with severe ischemic cardiomyopathy who were randomly assigned to receive either Vastarel (20 mg tid, n = 19) or placebo. At the end of the 2-month treatment period, all patients underwent echocardiography, both at rest and during infusion of low-dose dobutamine, and a cardiopulmonary exercise test. The resting ejection fraction in Vastarel treated patients increased from $33.1 \pm 4.5\%$ to $39.5 \pm 5.9\%$ ($P = 0.001$); left ventricular systolic volume decreased from 121.8 ± 9.2 mL to 110.2 ± 13 mL ($P = 0.003$); and the number of dysfunctional segments was reduced from 147 to 137. Low-dose dobutamine (5–20 μg/kg per min) improved contractility in 99 of 179 segments (a 30% increase relative to the initial study), compared with no significant changes in patients receiving placebo. In addition, the peak oxygen consumption increased significantly, from 16.4 ± 1.4 mL/kg per min to 18.9 ± 1.7 mL/kg per min in patients receiving active treatment. It was concluded that Vastarel improves resting contractile function, in addition to the contractile response to inotropic stimulation by low-dose dobutamine, in patients with severe ischemic dysfunction. These improvements suggest that the metabolic mode of action of Vastarel has a direct cytoprotective effect on myocardial cells, which carries potential prognostic implications in patients with heart failure.

## Effect of Vastarel on microcirculation and in diabetic patients

Diabetic patients are known to have structural abnormalities of the small vessels (microcirculation) affecting vasodilatory reserve, in addition to having epicardial coronary obstruction. Microcirculatory resistance is neither constant in time nor uniform in different perfusion areas. Di Girolamo et al [4] assessed the effect of Vastarel on myocardial microcirculation in patients with stable coronary artery disease and showed a significant reduction in defects in exercise stress thallium-201 scintigraphy, and an improvement in the ischemic threshold. The bene-

ficial effects were postulated to be from the reversal of cellular edema and extravascular compression of the coronary microvascular network brought about by Vastarel.

Diabetic patients are also more likely to have metabolic abnormalities such as impaired glycolysis, pyruvate oxidation, and lactate uptake, and greater dependency on fatty acids as a source of acetyl coenzyme A. Vastarel, with its specific metabolic action, is a suitable treatment for these patients. Fragasso et al [5] studied the short- and long-term specific beneficial effects of Vastarel in a small cohort of diabetic patients with severe ischemic dilated cardiomyopathy in whom it produced an improvement in left ventricular ejection fraction and fractional shortening after 2 weeks of treatment. This positive effect was maintained in the long term, after 6 months of treatment (*Figure 1*). Szwed et al [6] showed that diabetic patients with stable angina pectoris enjoyed the same level of benefits as nondiabetic individuals in having improvement in total exercise duration and time to 1 mm ST-segment depression, and significant decreases in weekly frequency of anginal episodes and weekly consumption of nitrate medication.

Effect of Vastarel on patients after myocardial infarction

Acute myocardial infarction results in profound myocardial ischemia and potential permanent loss
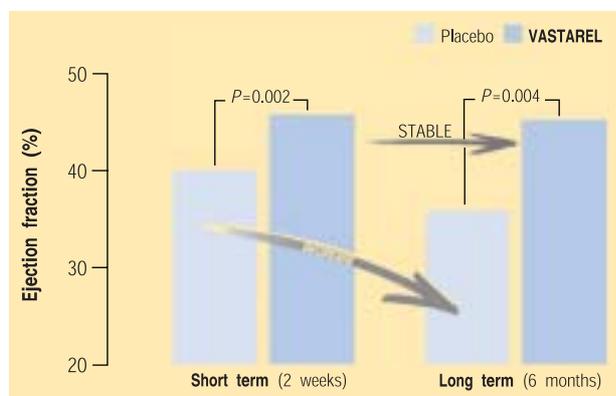
Figure 1. Effect of trimetazidine on left ventricular ejection fraction and fractional shortening in a cohort of diabetic patients with severe ischemic dilated cardiomyopathy, after 2 weeks and 6 months of treatment. (Reproduced from Fragasso et al [5], with permission.)

of myocytes and function. Reperfusion treatment remains the cornerstone of the treatment of these patients, but metabolic intervention seems a promising approach to lessen myocardial injury and limit infarct size. Infusion of glucose, insulin, and potassium – a form of metabolic intervention – has been studied and found to be associated with a reduction in infarct size and enhancement of functional recovery [7,8].

The Limitation of Infarct Size by Trimetazidine (LIST) study was a double-blind, randomized trial that included 94 patients who presented with a first episode of myocardial infarction with ST-segment elevation [9]. The patients were admitted within 6 hours of the onset of symptoms and had a totally occluded (TIMI grade 0 or 1) culprit artery that was adjudged to be amenable to percutaneous transluminal coronary angioplasty. The treatment regimen was an intravenous bolus dose of 40 mg Vastarel, followed by an infusion of 60 mg/day for 48 hours. In the Vastarel group, the return to baseline of the ST segment was achieved significantly earlier than in the placebo group ($P = 0.014$). In addition, there was a trend toward a less frequent exacerbation of ST-segment elevation immediately after reperfusion, which is a marker of reperfusion injury (23% compared with 42%; $P = 0.11$). Papadopoulos et al [10] showed that, in comparison with placebo, Vastarel significantly decreased the onset of reperfusion arrhythmias in patients who had undergone angioplasty [10].

For metabolic intervention treatment to work, it is imperative that myocardial viability remains preserved after myocardial injury. Vastarel has been shown to improve myocardial function after revascularization treatment in patients with ischemic cardiomyopathy who had demonstrable myocardial viability before operation. Ciavolella et al [11] studied 12 patients treated with Vastarel and reported a significant increase in tracer uptake, mainly in viable segments (proven on technetium-99m sestamibi single photon emission computed tomography and echocardiography) that showed improved myocardial function postoperatively.

Finally, a recent review by Marzilli has summarized all the available clinical evidence showing how a metabolic intervention with Vastarel can protect the heart from the deleterious consequences of ischemia (12).

Conclusions

Optimizing energy metabolism in the ischemic heart is a novel approach for the management of both ischemic heart disease and heart failure. The stimulation of myocardial glucose oxidation directly through the use of metabolically active agents, or indirectly through secondary inhibition of fatty acid oxidation, improves the production and utilization of energy at cellular level. These changes in cardiac metabolism are critical steps in affording benefit to a wide spectrum of patients, from those with stable angina to those with ischemic cardiomyopathy. Clinical findings with Vastarel MR, the first 3-ketoacyl coenzyme A thiolase inhibitor, have shown promise in such a metabolic interventional approach. ■

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Case Report

Imaging of endothelial dysfunction

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Abstract

Endothelial dysfunction is characterized by coronary vasoconstrictive responses to endothelium-dependent vasodilators and is considered to be an early phase of atherosclerosis. Various diagnostic techniques are available for the detection of endothelial dysfunction, such as coronary arteriography in combination with intracoronary ultrasonography and Doppler guidewire flow measurements, invasive venous occlusion plethysmography, functional magnetic resonance imaging, single-photon emission computed tomography, and positron emission tomography. Large studies have shown that pharmacological interventions, which are known to restore endothelial function, can prevent cardiovascular events. Early detection of this disorder is therefore of great clinical importance.
■ *Heart Metab.* 2004;22:33 – 36.

Keywords: Endothelial dysfunction, imaging, therapy

Case report

A 56-year-old woman was referred to our clinic because of typical anginal symptoms and exertional dyspnea. Her general physician had previously diagnosed mild hypertension and hyperlipidemia, for which she was successfully treated with an angiotensin-converting enzyme (ACE) inhibitor and statins. No additional cardiovascular risk factors were present. Her symptoms had started 1 year before her referral and were slowly progressive. On physical examination the pulse was 76 beats/min, and the blood pressure was 145/85 mm Hg. The patient was obese (body mass index 33 kg/m^2). Jugular venous pressure was 3 cm H_2O, and heart sounds were normal, without murmurs. Pulmonary and abdominal examinations were normal, and there was no peripheral edema. The electrocardiogram (ECG) (*Figure 1*) revealed marked abnormalities. In addition to an incomplete right bundle branch block, there was ST-segment depression with concomitant T-wave inversion in leads II, III, aVF, and V$_{4–6}$. Criteria for left or right ventricular hypertrophy were not met. Echocardiography was virtually impossible because

of a poor acoustic window. Magnetic resonance imaging (*Figure 2*), however, demonstrated a normal cardiac function and normal dimensions of the cardiac chambers; hypertrophy and valvular pathology were also ruled out. During exercise stress technetium-99m sestamibi single-photon emission computed tomography (SPECT), the patient had complaints of chest pain and her ECG revealed additional ST-segment depression of 1 mm in several leads. Surprisingly, the SPECT images were completely normal, both at rest and during stress. Quantitative perfusion determined by positron emission tomography (PET) using oxygen-15-labeled water, however, showed a relatively high resting perfusion (1.3 mL/min per mL) and an impaired hyperemic response to pharmacologically induced vasodilatation (2.4 mL/min per mL). Perfusion reserve, therefore, was also impaired (1.8). Interestingly, the impairment in perfusion reserve was evenly distributed throughout the left ventricle, explaining the false negative results of the SPECT images (see Comment section).

Pharmacological treatment was extended with aspirin and a β-blocker. Although the patient did

Case Report

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Figure 1. Electrocardiogram showing marked abnormalities.

Figure 2. An end-diastolic two-chamber view acquired with magnetic resonance imaging.
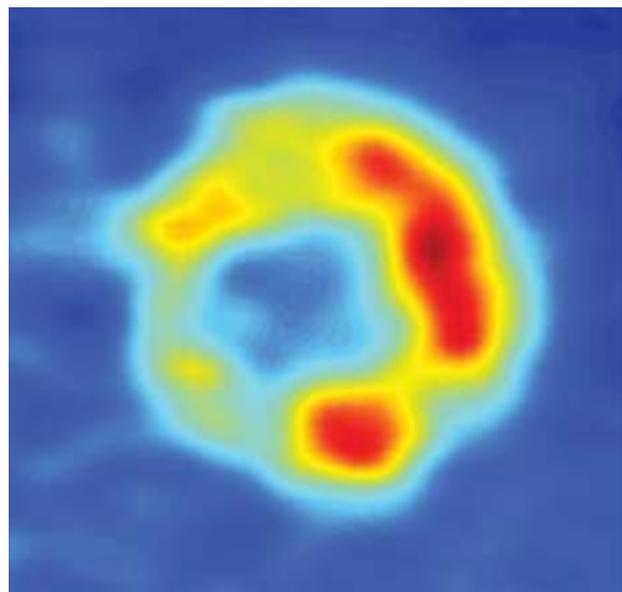

Figure 3. Post-exercise positron emission tomography using $[^{18}F]2$-fluoro-2-deoxyglucose (FDG) as a tracer in a short-axis view at the midventricular level. There is regional increased uptake of FDG in the anterolateral and inferior myocardium.

respond to medical treatment, she remained symptomatic (New York Heart Association Class II), even after treatment had been further extended with nitrates and a calcium antagonist. Coronary angiography was therefore performed and showed no epicardial stenosis in any of the coronary arteries. Quantitative measurements of coronary flow velocity by intracoronary Doppler guidewire confirmed the PET perfusion data, demonstrating a relatively high basal flow (30–35 cm/s) and impaired flow reserve (1.8–2.0) in each of the coronary arteries. These findings are suggestive of increased microvascular resistance. To evaluate whether the impairment of flow reserve indeed caused ischemia in this patient, an exercise stress $[^{18}F]2$-fluoro-2-deoxyglucose (FDG) PET scan was performed. *Figure 3* shows the image

obtained, clearly demonstrating an inhomogeneous distribution pattern of FDG. These results are indicative of myocardial ischemia in the areas of increased FDG uptake. Trimetazidine was added as antiischemic medical treatment. The patient is currently in a stable condition and her symptoms are no longer progressive.

Comment

Endothelial dysfunction is a prognostic factor for future cardiovascular events and is considered to be an early phase of coronary atherosclerosis [1]. Endothelial function has an important role in regulating thrombosis, thrombolysis, platelet, and leukocyte interactions, vascular tone, and coronary blood flow. Nitric oxide metabolism seems to be of particular importance in endothelial function, hence decreased production of nitric oxide characterizes endothelial dysfunction [2]. Early detection of endothelial dysfunction is important, because pharmacological, dietary, and lifestyle modifications can prevent cardiovascular events and revascularization procedures [3–5]. Several imaging techniques are available to detect endothelial dysfunction, such as coronary arteriography in combination with intracoronary ultrasonography and Doppler guidewire flow measurements, invasive venous occlusion plethysmography, functional magnetic resonance, SPECT, and PET [5]. Obviously, noninvasive imaging is preferred to invasive procedures.

SPECT with a perfusion tracer is noninvasive and widely available, but has an important limitation. As demonstrated in this particular case report, SPECT imaging represents the relative distribution of a tracer and therefore necessitates a normal reference area, leading to false negative results in the event of a diffuse reduction in perfusion. PET has the capability of quantifying perfusion in absolute terms, but is limited by its availability and high cost. Both techniques can also visualize metabolic processes such as glycolysis, using FDG. Endothelial dysfunction can eventually lead to ischemia, which is caused by limited blood supply in relation to demand; perfusion alone cannot truly identify ischemia. In ischemic myocardium, anaerobic glycolysis is increased, which leads to glycogen depletion. In the post-ischemic period, glucose uptake is enhanced in post-ischemic myocardium, in order to restore the glycogen pool [6]. This probably explains the regionally increased uptake of FDG tracer noted in this case report.

Intracoronary Doppler guidewire flow reserve measurements are invasive, but can be performed in the majority of cardiovascular clinical centers. In the absence of an epicardial stenosis, an impaired flow reserve suggests the presence of increased microvascular resistance, which is associated with endothelial dysfunction.

Once the diagnosis of endothelial dysfunction has been made likely, treatment can be initiated that allows restoration of endothelial function. Results of large trials provide evidence for a prognostic benefit from treatment with statins and ACE inhibitors [3,4]. The effects of these pharmacological agents seem to go beyond the reduction of cholesterol concentrations and decreasing blood pressure. Statins, among others, stimulate nitric oxide synthase, have anti-inflammatory effects, and reduce oxidative stress through their antioxidant properties [7]. In the Heart Outcomes Prevention Evaluation trial, the reduction in cardiovascular events with the ACE inhibitor, ramipril, was independent of the reduction in blood pressure [4]. Inhibition of the breakdown of bradykinin, together with antioxidative properties, are the proposed beneficial effects of ACE inhibitors on endothelial function [7]. More studies are needed to elucidate the exact effects of these agents on the endothelium.

Conclusion

Endothelial dysfunction is considered to be an early phase of atherosclerosis. Detection of this disorder is important, as pharmacological treatment can prevent cardiovascular events. A variety of invasive and noninvasive techniques are currently available for the detection of endothelial dysfunction.■

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Regulation of coronary perfusion

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Abstract

Many factors influence the regulation of coronary perfusion. They include metabolic, endothelial, humoral, autoregulatory, myogenic, extravascular compressive, and neural control mechanisms. The metabolite adenosine has a major influence on vasodilatation, and locally produced vasoactive substances such as nitric oxide also help to regulate myocardial blood flow. In addition, nitric oxide is implicated in autoregulation through pressure-sensitive ion channels. Neural control of coronary blood flow acts through direct neuronal stimulation or catecholamine release. These factors are discussed in detail in this article.
■ *Heart Metab.* 2004;22:37–41.

Keywords: Coronary perfusion, regulation, vasoactive, nitric oxide

Introduction

The coronary circulation supplies the myocardium with oxygen and substrates, and removes metabolic waste products. Cardiac contractile function requires aerobic metabolism and, as basal oxygen extraction is about 60%, an adequate increase in coronary blood flow is required to meet increased oxygen consumption. Coronary perfusion is regulated by a complex interplay of several factors that allows coronary blood flow to increase about 5-fold during strenuous exercise.

Changes in coronary vascular tone are essential for the adaptation of coronary blood flow to varying hemodynamic and metabolic demands [1]. Blood flow depends on both the aortic driving pressure and the resistance offered by the coronary bed. Several different control mechanisms regulate coronary vascular resistance; they include metabolic, endothelial, humoral, autoregulatory, myogenic, extravascular compressive, and neural factors. There is a heterogeneity in the response to the last two of these throughout the coronary circulation [1, 2], and it is worth considering the differing coronary vessels and compartments separately.

Coronary vasculature

The large epicardial coronary arteries are conductive vessels which do not contribute significantly to vascular resistance. Myogenic autoregulation of the vascular lumen occurs in these vessels, in response to alterations in aortic pressure. Modulation of coronary tone also occurs here, in response to flow-mediated endothelium-dependent vasodilatation, circulating vasoactive substances, and neural stimuli.

Myocardial oxygen extraction is virtually constant over a wide range of cardiac work and perfusion pressures. The resistive vessels match myocardial blood flow to variable myocardial energy requirements, and to myocardial demand when the coronary perfusion pressure varies. Coronary resistance is influenced both by extrinsic factors such as myocardial compression and by intrinsic factors such as tissue metabolism and neural and humoral mediators. Different mechanisms may account for the heterogeneity of the response of resistive vessels, such as different populations and subtypes of receptors for vasoactive substances [3] or variable metabolic pathways [4]. The resistive vessels have been separated into two general groups: prearteriolar and arteriolar [5, 6]. The arterioles ($<100\ \mu$m) respond to

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local tissue metabolism and maintain the extracellular environment within optimal biochemical limits for myocardial contractile function, modulated primarily by tissue oxygen tension. Prearteriolar vessels (100–350 μm) are influenced by coronary perfusion pressure and flow, myogenic tone, and neurogenic factors [6].

Figure 1 summarizes the factors influencing the regulation of coronary perfusion [7].

Quail et al [2] classified the regulatory factors into those acting from the adventitial aspect of coronary smooth muscle (eg, phasic myogenic compressive forces and autonomic neurotransmitters), and those acting from the luminal aspect (eg, endothelium-dependent or -independent vasodilator or constrictor substances), plus the myogenic properties of the vascular smooth muscle itself, responsible for auto-regulation [2].

Metabolic control

Arterioles are directly exposed to the effects of the myocardial metabolites, which diffuse into the interstitial space. The vasodilator metabolites cause smooth muscle cell relaxation and vasodilatation, and thus increased flow. Adenosine is believed to be the major substance that influences metabolically induced coronary vasodilatation [8]; it has also been investigated most extensively. It is formed by 5'nucleotidase from adenosine monophosphate (AMP), which itself arises from adenosine triphosphate (ATP). It diffuses into the interstitial space, where it can induce arteriolar dilation and re-enter the myocardial cell. It is either phosphorylated to AMP by adenosine kinase, or deaminated to inosine monophosphate by adenosine deaminase, or it can enter the capillaries and leave the tissue.

Nitric oxide also is implicated in metabolic control. There are two known stimuli for release of nitric oxide, namely hypoxia and flow-mediated dilatation. It is believed that hypoxia initiates hyperaemia, and flow-mediated dilatation sustains and amplifies it.

Oxygen tension, acid–base balance, potassium, osmotic pressure, and ATP-sensitive potassium channels also contribute to the metabolic regulation of flow.

Endothelium-mediated regulation

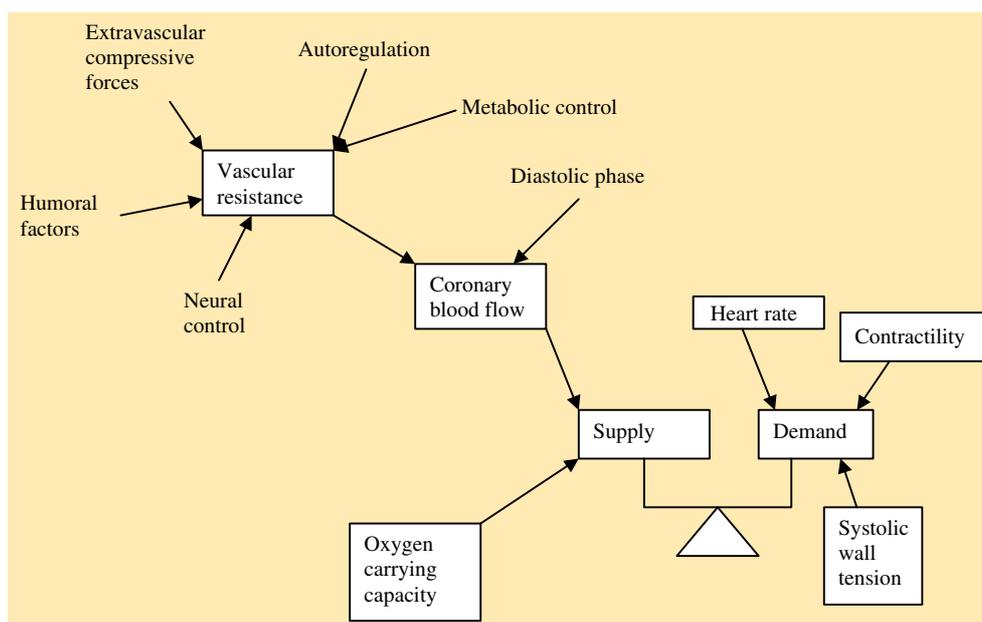The arterial endothelium comprises cells resting on a basement membrane, which have autocrine, para-

Figure 1. Factors influencing the regulation of coronary perfusion. (Adapted from Braunwald et al [7], with permission.)
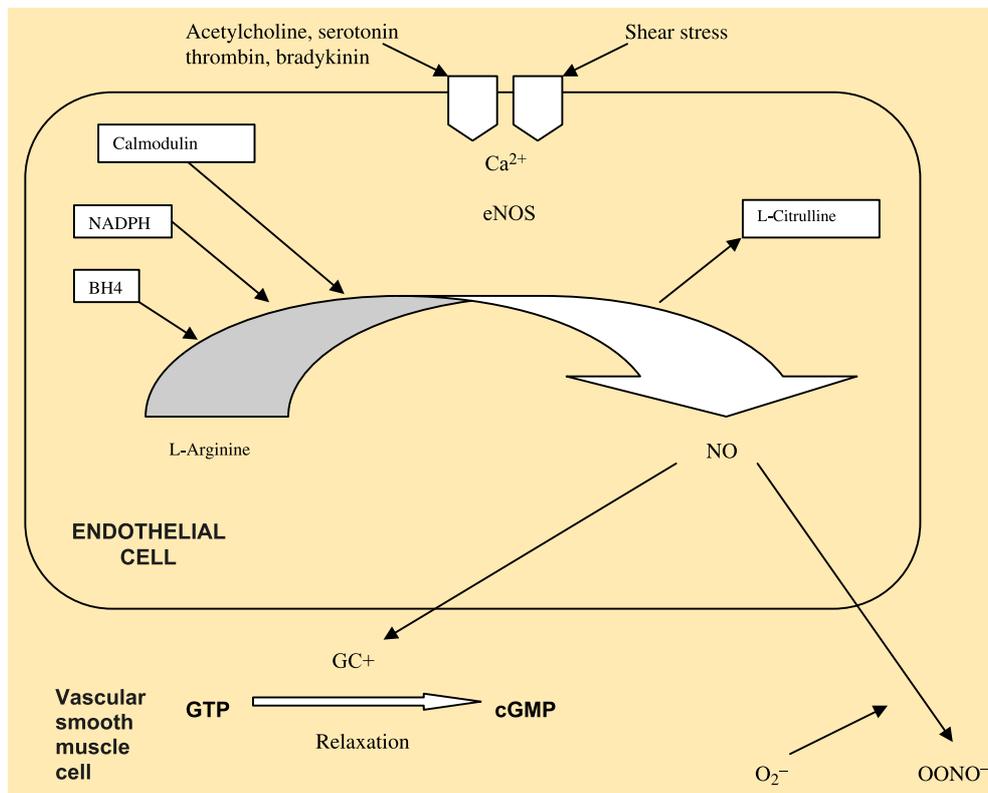
Figure 2. Production of nitric oxide (NO) by the action of endothelial nitric oxide synthase (eNOS) on L-arginine. Cofactors such as tetrahydrobiopterin (BH4), calmodulin, and reduced nicotinamide adenine dinucleotide phosphate (NADPH) are involved in the process. Stimulation of eNOS by vasodilator agonists or shear stress is mediated by an increase in intracellular calcium (Ca^{2+}). Nitric oxide may be broken down by free radicals (O_2^-), producing peroxynitrite ($OONO^-$), which is vasoactive. Nitric oxide acts on vascular smooth muscle cells to cause relaxation by activating guanylate cyclase (GC), thereby increasing intracellular cyclic guanosine monophosphate (cGMP). (Adapted from Braunwald et al [7], with permission.)

crine, and endocrine functions [9]. This monolayer of endothelial cells has a crucial role in the regulation of coronary vasomotor tone through the elaboration of potent endothelium-derived vasoactive factors formed locally [10]. The endothelial cells also regulate inflammation, thrombosis, fibrinolysis, and cell–cell interactions [11]. Endothelial cells produce and release both vasodilator and vasoconstrictor factors. One of the factors modulating vasodilatation is nitric oxide [12], produced from L-arginine by nitric oxide synthetase [13] (*Figure 2*).

Nitric oxide may be released in response to a variety of different stimuli: flow (shear stress), platelet-derived products (ADP, thrombin, serotonin), and vasoactive agents (bradykinin, histamine, norepinephrine, substance P, vasopressin). Vasoconstrictor substances such as endothelin may override the normal vasomotor tone associated with endothelium-dependent vasodilatation in both pathological and physiological states [14]. In the human coronary circulation, infusion of an inhibitor of nitric oxide production causes a small reduction in blood flow in normal arteries [15], indicating a basal release of nitric oxide to maintain resting flow. Studies in the peripheral circulation in an animal model showed that nitric oxide activity is greatest in vessels larger than 100 μm in diameter [16], which are under the most shear stress, the major determinant of nitric oxide release. Studies confirm the importance of nitric oxide in modulating microvascular flow by dilating the prearterioles by between 100 and 300 μm, thus preserving the vasodilator potential of

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the arterioles smaller than 100 μm [17]. In athero-sclerosis, a loss of this endothelium-dependent mechanism for microcirculatory regulation could account for changes in vasomotor tone at the prearteriolar level, upstream from the potent meta-bolic vasodilator stimuli of hydrogen ions and low tissue oxygen tension.

Increased release of nitric oxide is seen as a result of stimuli such as an increase in blood pressure or a decrease in the partial pressure of oxygen, or also secondary to the action of acetylcholine, ADP, ATP, bradykinin, or histamine [18].

Autoregulation

There is a broad range of arterial pressures over which coronary autoregulation can occur, permitting sus-tained and constant coronary blood flow. However, this autoregulation may fail at extremes of arterial pressure change [19]. There are upper and lower limits to the autoregulatory range, but they are not reached in physiological conditions.

There are two proposed mechanisms of autoregu-lation. First, nitric oxide is believed to be involved through the ability of the endothelium to sense changes in perfusion pressure through pressure-sensitive ion channels. Inhibition increases the lower autoregulatory threshold by about 15 mm Hg. Myo-genic control plays a small part in autoregulation, as the smooth muscle in the artery wall contracts in response to increased intraluminal pressure.

Neural control

The autonomic nervous system acts to modulate coronary blood flow through direct neuronal stimula-tion (vessels >100 μm) or by the release of catecho-lamine [20]. It has been demonstrated previously that selective α_2-adrenergic activation may induce en-dothelium-dependent vasodilatation in isolated canine epicardial arteries [21]. In the open-chest dog model, α_1- and α_2-adrenergic activation con-stricted prearterioles and arterioles, respectively. Inhibition of nitric oxide synthesis unmasked addi-tional vasoconstriction by α_1-adrenergic activation in arterioles and α_2-adrenergic activation in the prearter-ioles [22]. This implies that the release of nitric oxide, induced by the shear stress of increased coronary flow, opposes α-adrenergic vasoconstriction, thus limiting the potential reduction in myocardial perfu-sion during augmented sympathoadrenal drive. In pathological states, endothelial dysfunction may lead to unopposed α-adrenergic vasoconstriction and subsequent prearteriolar resistive vessel dysfunction.

Conclusion

It is clear that the regulation of coronary perfusion is a complex process, and relies on the integration of the factors that are described in this article. It is likely that further research into these factors could improve our treatment strategies in patients with coronary artery disease in the future. ■

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Abstracts and commentaries

G972R IRS-1 variant impairs insulin regulation of endothelial nitric oxide synthase in cultured human endothelial cells

Federici M, Pandolfi A, De Filippis EA, et al. *Circ Res.* 2004;109:399–405

Endothelial dysfunction – ie, reduced nitric oxide availability, a pivotal step in the pathogenesis of atherosclerosis – is a feature of insulin-resistant states such as type 2 diabetes, obesity, and hypertension. Impaired insulin-mediated vasodilation might contribute to vascular damage in insulin-resistant states. The aim of this work was to investigate insulin regulation of nitric oxide synthesis in human umbilical vein endothelial cells (HUVECs) carrying an insulin receptor substrate-1 (*IRS-1*) gene variant known to be associated with impaired activation of insulin signaling downstream of IRS in transfected cells. The results demonstrate that genetic impairment of the IRS-1/phosphatidylinositol 3-kinase [PI(3)K]/phosphoinositide-dependent protein kinase-1 (PDK-1)/Akt (also known as protein kinase B) insulin signaling cascade determines impaired insulin-stimulated nitric oxide release. They also suggest that the G972R-*IRS-1* gene polymorphism, through a direct impairment of Akt/endothelial nitric oxide synthase (eNOS) activation in endothelial cells, may contribute to the genetic predisposition to develop endothelial dysfunction and cardiovascular disease.

Commentary

Insulin resistance is associated with atherosclerosis and coronary artery disease. Both the metabolic alterations occurring in the insulin resistant state and the possible detrimental effects of hyperinsulinemia have been proposed to explain this association. Insulin promotes vasodilation and increases blood flow, thus participating in the regulation of hemodynamic homeostasis. Insulin signaling is mediated by complex multiple cascade pathways character-ized by spatial and temporal features. It is initiated by its binding to the insulin receptor. This activates the tyrosine kinase activity of the receptor, leading to its autophosphorylation and to the subsequent phosphorylation of IRS-1. IRS-1 phosphorylation leads to interaction of IRS-1 with the PI(3)K p85 regulatory subunit. PI(3)K then produces phosphatidylinositol 3,4,5-P3 and 3,4-P2, which bind to the pleckstrin homology domain of at least two different protein kinases, namely PDK-1 and Akt (protein kinase B). It has been demonstrated that IRS-1 and PDK-1 are required for the insulin-stimulated production of nitric oxide in endothelial cells. The authors in the present work used HUVECs from carriers of the *IRS-1* gene G972R variant to determine whether a polymorphism known to impair insulin action might reduce the ability of insulin to activate the signaling pathway that regulates the activity and expression of eNOS. In HUVECs naturally expressing the G972R-*IRS-1* variant, they observed cell-specific impairment of insulin action, as revealed by defective insulin-stimulated activation and expression of eNOS. In the cells carrying this G972R-*IRS-1* variant, the IRS-1/ PI(3)K/PDK-1/Akt insulin signaling cascade was impaired, as manifested by reduced IRS-1-associated PI(3)K activity and reduced insulin-stimulated Akt phosphorylation. This resulted in both insulin-stimulated expression of eNOS and impaired activation of eNOS. The results demonstrate a potential mechanism by which insulin resistance can be involved in vascular dysfunction and hence abnormalities. These data might have relevant clinical implications. Previous studies have indeed shown that the frequency of the G972R-*IRS-1* polymorphism is significantly greater in patients with angiographic evidence of coronary artery disease than in control individuals. When adjusted for other risk factors, the relative risk of coronary artery disease associated with the G972R-*IRS-1* polymorphism was 2.93-fold greater than that in wild-type individuals, and it increased to 6.97-fold in obese individuals and to 27.3-fold in those with clinical features of insulin resistance syndrome [1].

REFERENCE

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Danielle Feuvray

Seven-year outcome in the RITA-2 Trial: coronary angioplasty versus medical therapy
J Am Coll Cardiol. 2003;42:1161–1170

Advances in medical therapy for the treatment of stable angina have, in the past 10 to 15 years, been of substantial importance. If, on diagnosis we utilize all the evidence-based treatments – statins, aspirin, β-blockers and angiotensin-converting enzyme inhibitors – there is a potential 75% reduction in risk for significant cardiac events for at least 5 years. Often overlooked is the low event rate in patients with stable angina, for whom there is a death or nonfatal myocardial infarction rate of 2–3% per year. This means that, with improving medical therapy, there is time to optimize management medically, assess the patient for risk by means of exercise electrocardiography and echocardiography, and then, in those not at risk, decide on conservative or interventional treatment on the basis of symptoms and quality of life. The medical control of symptoms should include conventional hemodynamic agents, in addition to the metabolic approach using trimetazidine.

The findings of the Randomised Intervention Treatment of Angina-2 Trial confirm the importance of medical treatment – albeit suboptimal by today's standards. It was a randomized trial of patients with stable angina, comparing medical treatment (n = 514) with coronary angioplasty (n = 504). Both arms can be criticized for low use of both statins and stents, reflecting the era when the trial commenced; however, bearing in mind that caveat, the results are of great interest. At 7 years follow-up, death or myocardial infarction had occurred in 73 (14.5%) of the patients who underwent percutaneous transluminal coronary angioplasty and in 63 (12.3%) of those who received medical treatment, with 43 deaths in each group, only 41% of which were cardiac-related. Once again, the low overall event rate has thus been confirmed and the importance of treating the patient, not just the anatomy, clearly emphasized. With no evidence that coronary angioplasty, with or without a stent, reduces myocardial infarction or death rates, the role of percutaneous coronary intervention in patients with stable angina is that of symptom relief. Mortality rates are related to baseline risk, which can be defined by noninvasive screening. Symptom relief is related to the optimal use of medical treatment and adequate dose titration of hemodynamic agents (eg, β-blockers), combined with metabolic therapy such as trimetazidine. Medical treatment can be safely given time to be effective and, if symptoms persist, intervention is then recommended.

Graham Jackson

Arterial stiffness, wave reflections, and the risk of coronary artery disease
Circulation. 2004;109:184–189

Increased arterial stiffness, determined invasively, has been shown to predict a higher risk of coronary atherosclerosis. However, invasive techniques are of limited value for screening and risk stratification in larger patient groups. We prospectively enrolled 465 consecutive, symptomatic men undergoing coronary angioplasty for the assessment of suspected coronary artery disease. Arterial stiffness and wave reflections were quantified noninvasively using applanation tonometry of the radial artery with a validated transfer function to generate the corresponding ascending aortic pressure waveform. Augmented pressure (AP) was defined as the difference between the second and the first systolic peak, and augmentation index (AIx) was AP expressed as a percentage of the pulse pressure. In univariate analysis, a higher AIx was associated with an increased risk for coronary artery disease (odds ratio [OR], 4.06 for the difference between the first and the fourth quartile [1.72 to 9.57; $P < 0.01$]). In multivariate analysis, after controlling for age, height, presence of hypertension, high-density lipoprotein cholesterol, and medications, the association with coronary artery disease risk remained significant (OR, 6.91; $P < 0.05$). The results were exclusively driven by an increase in risk with premature vessel stiffening in the younger patient group (up to 60 years of age), with an unadjusted OR between AIx quartiles I and IV of 8.25 ($P < 0.01$) and

a multiple-adjusted OR between these quartiles of 16.81 ($P < 0.05$). We conclude that AIx and AP, noninvasively determined manifestations of arterial stiffening and increased wave reflections, are strong, independent risk markers for premature coronary artery disease.

Commentary

The reflection of pressure waves from the peripheral circulation back towards the aorta modulates the shape of the central aortic pressure waveform. The stiffer the aorta and large arteries, the greater the velocity at which the pressure wave travels, both anterograde and retrograde, and hence the earlier during systole the returning wave reaches the aorta. In addition, the stiffness of the small to medium-sized muscular arteries determines the total amount of reflection. Thus a large premature reflected wave suggests that the aorta and large arteries are stiff, whereas the small and medium-sized arteries have high vascular tone. The central aortic waveform can therefore be used as a global measure of large- and small-vessel ''health.'' The central aortic pressure can only be measured directly, invasively. However, the waveform can be derived by transforming the shape of the peripheral pulse pressure, which in turn can be derived by applanation tonometry.

Weber and colleagues used applanation tonometry and standard measures that combine reflected pulse wave amplitude and prematurity to determine prospectively the arterial stiffness in male patients undergoing diagnostic coronary angiography. Of the 465 patients examined, 59 did not have coronary artery disease. This group were significantly younger and taller, and had a lower prevalence of hypertension and antihypertensive medication. Surprisingly, on treatment, there was little difference in systolic blood pressure between groups, but diastolic pressure was greater – and thus pulse pressure lower – in those without coronary artery disease. The most persuasive finding was that, even when these factors where controlled by multivariate analysis, early and large pulse wave reflection predicted the presence of coronary artery disease. This was most obvious in patients younger than 60 years, 43% of whom in the lower quartile of measures of early reflection had normal coronary arteries, compared with just 8% in the upper quartile. Furthermore, it is likely that applanation tonometry would have been even more highly discriminatory if it were not for the fact that there was a greater prevalence of use of nitrate and angiotensin-converting enzyme inhibitors in those patients with coronary artery disease. These medications reduce both pulse wave velocity and reflected amplitude. The findings reinforce those from other studies that suggest pulse wave reflection is a useful noninvasive measure of vascular disease.

M. Marber

Glossary

5-HT(1D)-serotonin receptor

Serotonin (5-hydroxytryptamine or 5-HT) is a transmitter in the central nervous system, and also functions in the periphery as a ubiquitous hormone involved in vasocontriction and platelet function. Serotonin acts on a variety of serotonin receptors, one of these being the 5-HT1D receptors.

Autacoids

Autacoids are organic substances produced in one cell type that act either on the same cell, or a cell nearby to produce a biological effect. Nitric oxide (NO) or prostaglandins are examples of autacoids.

Calmodulin

Calmodulin is an important molecule that binds calcium and stimulates the activity of calmodulin-dependent kinases. Calmodulin mediates many important reactions in the cell, including excitation contraction coupling of muscle cells.

Caveolin

Caveolie are invaginations in the plasma membrane of cells that represent subcompartments of the plasma membrane. Caveolins are caveolie coat proteins. Recent interest has focussed on the role of G-proteins associated with caveolins as a mechanism for transmembrane signalling.

Cyclic GMP

Cyclic guanylate monophosphate (cyclic GMP) is produced from guanylate triphosphate (GTP) via the enzyme guanylate cyclase. Cyclic GMP has numerous actions as an intracellular signalling molecule, including relaxation of smooth muscle. The vasodilatory effect of NO on smooth muscle is mediated by the production of cyclic GMP by guanylate cyclase.

Epoxyeicosatrienoic acids

Epoxyeicosatrienoic acids (EETs), which are synthesized from arachidonic acid by cytochrome P450 epoxygenases, function primarily as autocrine and paracrine effectors in the cardiovascular system and kidney. The EETs have diverse actions, including somatostatin, insulin and glucagon release from the pancreas. They also modulate ion transport and gene expression, producing vasorelaxation, as well as anti-inflammatory and pro-fibrinolytic effects.

G-proteins

G-proteins refer to a group of guanylate triphosphate (GTP) binding proteins that are crucial in linking numerous types of receptor to their subcellular signalling pathways. An example of this is the ß-adrenergic receptor, which is coupled to adenylate cyclase via a G-protein. Receptors that are coupled to the G-protein family are called G-protein coupled receptors.

Geranylgeranyl-pyrophosphate

Geranylgeranyl pyrophosphate (GGPP) is an isoprenoid that is a precursor for numerous molecules essential for cellular function. GGPP also acts as a substrate in isoprenylation reactions. GGPP is produced from farnesyl-PP, which is produced from geranyl-PP, an intermediate in the cholesterol synthetic pathway. Inhibition of GGPP production, using a geranylgernayl transferase inhibitor can inhibit vascular smooth muscle proliferation. A similar effect can be observed by HMG-CoA reductase inhibition, which inhibits the production of mevalonate, which eventually can go on to produce geranyl-PP.

Hydrobiopterin

Tetra-hydrobiopterin is produced by the reduction of dihydrobiopterin, catalyzed by the enzyme, dihydrofolate reductase. Tetra-hydro-

biopterin is an essential cofactor for nitric oxide (NO) formation.

L-arginine

L-arginine is an amino acid. An important function of L-arginine is as a substrate for nitric oxide synthase, which produces nitric oxide (NO). NO is a potent vasodilator of smooth muscle.

Proteins of the Rho-family

The Rho-family of proteins are proteins involved in cellular signalling. An example of this is Rho A, which is involved in vascular smooth muscle proliferation. Platelet-derived growth factor can increase Rho A protein. Rho kinase plays an important role in this process as an effector of Rho A. The RhoA/Rho kinase pathway can mediate calcium sensitization in vascular smooth muscle.

Substance P

Substance P is a tachykinin and physiologically acts as a neurotransmitter and neuromodulator in the nervous system. Pathologically, it can also trigger malignant cells to release cytokines and increase cell proliferation rates.

Thromboxane A_2

Thromboxane A_2 is a product of the cycloxygenase pathway of arachidonic acid metabolism. The production of PGH2 from arachidonic acids by cycloxygenase can be used for a number of different eicosanoid products, including the production of prostaglandins. Metabolism of PGH2 by thromboxane synthase, which is abundant in lung and platelets, results in the production of thromboxane A_2. Thromboxane A_2 has a variety of biological effects, including vasoconstriction and promotion of platelet aggregation.