

ENDOTHELIAL DYSFUNCTION AND TYPE 2 DIABETES

Part 1: physiology and methods for exploring the endothelial function

B. GUERCI (1), A. KEARNEY-SCHWARTZ (2), P. BÖHME (1), F. ZANNAD (2), P. DROUIN (1, 2)

SUMMARY - Coronary artery, cerebrovascular and peripheral vascular disease, are the principal causes of morbidity and mortality in type 2 diabetes mellitus. The accelerated macrovascular disease in type 2 diabetes mellitus is due partly to the increased incidence of cardiovascular risk factors, such as hypertension, obesity and dyslipidemia. Advanced glycation end products, glycoxidised and oxidized low-density lipoproteins and reactive oxygen species linked to hyperglycemia have all been identified in type 2 diabetes mellitus and could accelerate macroangiopathy. Hence, the resistance to insulin is an additional independent risk factor, in association with oxidant stress, dyslipidemias, and prothrombic/hypofibrinolytic states.

The endothelium is a major organ involved by cardiovascular risk factors, such as hypercholesterolemia, hypertension, inflammation, ageing, postmenopausal status, and smoking. Changes in endothelium function may lead to the coronary artery circulation being unable to cope with the increased metabolism of myocardial muscle independently of a reduced coronary artery diameter. The way endothelial function is altered in diabetic patients is not yet fully understood, but the loss of normal endothelial function could be involved in the pathogenesis of diabetic angiopathy, as endothelial dysfunction is associated with diabetic microangiopathy and macroangiopathy. Finally, recent reports indicate that an improved metabolic control in diabetic patients, whatever the treatment used, is associated with near normalization or restoration of normal endothelial function.


Key-words: type 2 diabetes mellitus, diabetic angiopathy, endothelium, endothelial function, hyperglycemia, oxidant stress, hyperlipidemia, insulin, insulin resistance.

RÉSUMÉ - Dysfonction endothéliale et diabète de type 2. Partie 1: Physiologie et méthodes d'exploration de la fonction endothéliale.

Les maladies cardiovasculaires constituent la cause principale de morbidité et de mortalité au cours du diabète de type 2. La raison de cette macroangiopathie accélérée chez les diabétiques de type 2 est expliquée en partie par l'incidence accrue d'hypertension artérielle, d'obésité et de dyslipidémies, facteurs de risque cardiovasculaire. Le processus de glycation non-enzymatique des protéines, l'oxydation et la gluco-oxydation des lipoprotéines plasmatiques, ainsi que l'hyperproduction d'espèces oxygénées réactives largement décrits au cours du diabète de type 2 sont liés à l'hyperglycémie et interviennent dans le développement de la macroangiopathie. Le syndrome d'insulinorésistance pourrait constituer un facteur de risque indépendant supplémentaire, et est associé au stress oxydatif, aux dyslipidémies, et aux phénomènes prothrombotiques et/ou hypofibrinolytiques.

Il est clairement établi que l'activité des cellules endothéliales est altérée au cours de l'hypercholestérolémie, l'hypertension artérielle, les phénomènes inflammatoires, pendant la période ménopausique et chez les sujets âgés et les fumeurs. Les modifications de l'endothélium peuvent conduire à une inadaptation de la circulation coronaire liée à un métabolisme accru du muscle cardiaque indépendamment de toute réduction du diamètre de la lumière artérielle. Les mécanismes en cause dans la dysfonction endothéliale observée au cours du diabète restent à élucider, mais la perte de la fonction normale de l'endothélium pourrait être impliquée dans le développement de l'angiopathie diabétique, car elle est présente chez les patients atteints de micro- et macroangiopathie diabétiques. Enfin, plusieurs auteurs ont récemment démontré que la prise en charge du déséquilibre métabolique du patient diabétique de type 2 s'accompagne, quel que soit le traitement mis en place, d'une amélioration, voire d'une normalisation de la fonction endothéliale.

Mots-clés : diabète de type 2, angiopathie diabétique, endothélium, fonction endothéliale, hyperglycémie, stress oxydant, hyperlipidémie, insuline, insulinorésistance.

 : B. Guerci, Service de Diabétologie, Maladies Métaboliques et Maladies de la Nutrition, Hôpital Jeanne d'Arc, CHU de Nancy, B.P. 303, 54201 Toul Cedex.
E-mail: b.guerci@chu-nancy.fr
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(1) Service de Diabétologie, Maladies Métaboliques et Maladies de la Nutrition, Hôpital Jeanne d'Arc, CHU de Nancy, B.P. 303, 54201 Toul Cedex.

(2) Centre d'Investigation Clinique, INSERM/CHU de Nancy, Hôpital Jeanne d'Arc, CHU de Nancy, B.P. 303, 54201 Toul Cedex.

The endothelium is the cell layer that lines blood vessels. For many years, it was believed to be a semipermeable barrier between the blood and the interstitium, facilitating the exchange of water and small molecules. But recent experiments have shown that the endothelium also participates in metabolic, synthetic and regulatory pathways [1]. The main effect of stimulating the endothelium is vasodilation in healthy people, and the local control of the vasculature depends on a balance between dilators and constrictors [2]. A healthy endothelium is also antiatherogenic, as it is able to inhibit platelet aggregation and adhesion, smooth muscle cell proliferation and leucocyte adhesion [3].

REGULATORY FUNCTIONS OF THE VASCULAR ENDOTHELIUM

Vasoregulation

The integrity of endothelium is needed to maintain the balance between vasodilation and vasoconstriction, and so preserve a sufficient vascular diameter for the satisfactory perfusion of the cardiovascular system. The endothelium is responsible for the short term regulation of this vascular tone. It produces vasodilator substances, such as nitric oxide (NO) (which was previously called endothelium-derived relaxing factor (EDRF)), prostacyclin (PGI₂) and endothelium-derived hyperpolarizing factor (EDHF). It also produces vasoconstrictor substances, such as endothelin-1, thromboxane A₂ (TXA₂) and prostaglandin H₂ (PGH₂). This vasoregulation is under the control of biochemical and mechanical stimuli (Fig. 1).

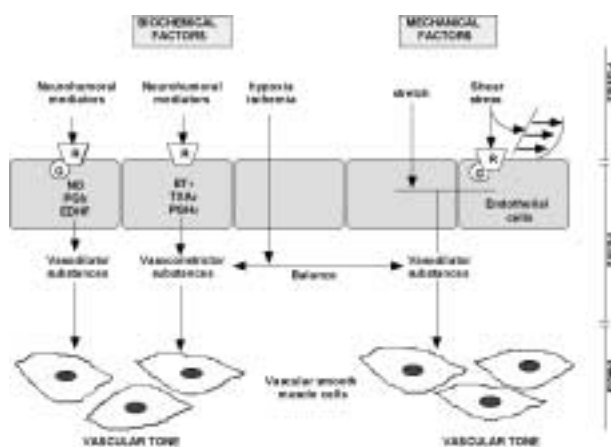


FIG. 1. Vasoregulation of the healthy endothelium. NO: nitric oxide, PGI₂: prostacyclin, EDHF: endothelium-derived hyperpolarizing factor, ET₁: endothelin-1, PGH₂: prostaglandin H₂, TXA₂: thromboxane A₂. ⊕ protein G, ⊖ receptor.

Vasodilators secreted by the endothelium (Fig. 2)

Nitric oxide (NO)

Since the demonstration by Furchgott and Zawadzki (1980) that the endothelium is essential for the relaxation of the isolated rabbit aorta in response to acetylcholine, the biological role of the endothelium and of NO has been extensively investigated in both animals and humans. NO is the best characterized and probably the most important vasodilator. NO is produced in response to a variety of stimuli by the oxidation of L-arginine by the NADPH-dependent enzyme, nitric oxide-synthase (NOS) [4]. Its production leads to physiological vasodilation and the relaxation of smooth muscle cells. This effect is mediated by protein G. There are three isoforms of NOS, all of which transform L-arginine into nitric oxide and L-citrulline; eNOS (endothelial NOS), iNOS (inducible NOS), and nNOS (neuronal NOS). Two of them (eNOS and nNOS) are calcium-dependent. The eNOS is located in the plasmalemma caveola of endothelial cells, close to caveolin-1, a protein that inhibits the enzyme activity of eNOS [5]. The nitric oxide acts on smooth muscle cells by stimulating guanylate cyclase

ABBREVIATIONS

- NO: nitric oxide
- EDRF: endothelium derived relaxing factor
- PGI₂: prostacyclin
- EDHF: endothelium-derived hyperpolarizing factor
- TXA₂: thromboxane A₂
- PGH₂: prostaglandin
- NOS: nitric oxide synthase
- cGMP: cyclic guanosine monophosphate
- ET₁: endothelin 1
- COX: cyclooxygenase
- cAMP: cyclic adenosine monophosphate
- Ach: acetylcholine
- ECE: endothelin-converting enzyme
- EDCFs: endothelium-derived contracting factors
- ADP: adenosine diphosphate
- ATP: adenosine triphosphate
- 5-HT: 5-hydroxytryptamin or serotonin
- AT-I: angiotensin I
- AT-II: angiotensin II
- ACE: angiotensin-converting enzyme
- ICAM: intercellular adhesion molecule
- VCAM: vascular cell adhesion molecule
- PECAM: platelet/endothelial cell adhesion molecule
- NF-κB: nuclear factor-kappa B
- TNFα: tumor necrosis factor-α
- IL-1: interleukin-1
- t-PA: tissue-type plasminogen activator
- PAI-1: plasminogen activator inhibitor-1
- vWF: von Willebrand factor
- PAF: platelet activating factor
- ELAM: endothelial leukocyte adhesion molecule
- L-NMMA: N^G-monomethyl-L-arginine
- L-NAME: N^G-nitroarginine methyl ester
- PDGF: platelet-derived growth factor
- VEGF: vascular endothelium growth factor
- TGF-β: transforming growth factor β

and by increasing the intracellular concentration of cyclic guanosine monophosphate (cGMP). The cGMP decreases the intracellular Ca^{2+} concentration causing vasorelaxation [6]. Nitric oxide also inhibits platelet aggregation by a mechanism dependent on cGMP, having an antithrombotic effect. Finally, NO also inhibits the proliferation of smooth muscle cells [7], the synthesis of adhesion molecules, and antagonises endothelin-1 (ET_1) [8]. Nitric oxide has a low molecular weight, diffuses rapidly and has a very short half life (a few seconds). It is thus an ideal tool for adapting the vasculature to changes in blood flow and allows instant changes in arterial diameter to cope with blood flow and shear stress.

Prostacyclin

Another major vasodilator is prostacyclin, which is produced from arachidonic acid by the enzymes cyclooxygenase (COX) and prostacyclin synthase. Its release may be stimulated by bradykinin and adenine nucleotides. Prostacyclin acts by stimulating adenylate cyclase and by increasing intracellular cyclic adenosine monophosphate (cAMP). Like nitric oxide, prostacyclin is a potent vasodilator with a short half life, and acts in both the systemic and pulmonary circulations [9]. Finally, prostacyclin plays a key role in the interaction between the endothelium and platelets by limiting the development of thrombi.

Endothelium-derived hyperpolarizing factor (EDHF)

Stimulation of the normal endothelium by acetylcholine (ACh) also produces hyperpolarization of the underlying smooth muscle cells hence vasorelaxation. This diffusible relaxing and hyperpolarizing substance, distinct from nitric oxide or prostacyclins and designated endothelium-derived hyperpolarizing factor (EDHF), is secreted by the endothelium, and contributes to endothelium-dependent relaxations by opening K^+ /ATP dependent channels in the vascular smooth muscle [10].

Vasoconstrictors secreted by the endothelium (Fig. 2)

Endothelin

This 21 amino acids peptide is an extremely powerful vasoconstrictor. There are three isoforms of endothelin, but isoform 1 (ET_1) only, is released from human endothelial cells [11, 12]. The production of endothelin-1 from big-endothelin is catalysed by endothelin-converting enzyme (ECE); it is released in response to hypoxia and noradrenalin [13]. The biological actions of endothelin are mediated by two distinct G-protein-coupled receptor subtypes (ET_A and ET_B), located in smooth muscle cells. Such activation causes an increase in intracellular calcium leading to contraction. ET_1 can also interact with ET_B receptors on endothelial cells, triggering the release of vasodilators (NO, PGI_2). ET_1 has a short half life and is present in healthy subjects at low concentrations. ET_1 is involved in counter-regulation for preserving peripheral resistance. Hopfner *et al.* have described its role in the cardiovascular system [14]. ET_1 also has an antimitotic effect on smooth muscle and endothelial cells.

Endothelium-derived contracting factors (EDCFs)

Under certain conditions, the endothelium also synthesizes and releases EDCFs, causing endothelium-dependent contractions [3]. These EDCFs include vasoconstrictor prostanoids such as prostaglandin H_2 and thromboxane A_2 , which activate specific receptors on the vascular smooth muscle. Superoxide anions may act as contracting factor by scavenging NO. The stimuli for EDCFs production are hypoxia, blood pressure, and variety of neurohumoral mediators.

Stimuli

Neurohumoral factors

The neurohumoral mediators (acetylcholine, bradykinin and histamine), hormones (catecholamines, vasopressin), and substances derived from platelets (adenosine diphosphate and serotonin) and thrombin (T) cause the release of endothelium-derived relaxing or contracting factors by activating specific endothelial receptors, inducing changes in the vascular tone.

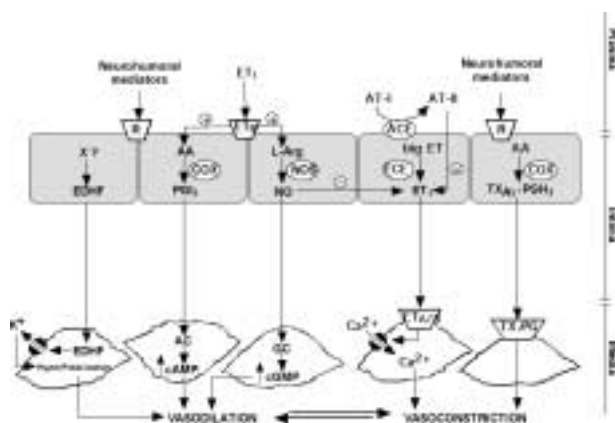


FIG. 2. Vasodilators and vasoconstrictors secreted by the endothelium. NO: nitric oxide, NOS: nitric oxide synthase, ET_1 : endothelin-1, ECE: endothelin-converting enzyme, PGI_2 : prostacyclin, COX: cyclooxygenase, L-Arg: L-arginine, EDHF: endothelium-derived hyperpolarizing factor, X?: unknown, cGMP: cyclic guanosine monophosphate, cAMP: cyclic adenosine monophosphate, PGH_2 : prostaglandin H_2 , TXA_2 : thromboxane A_2 , AT-I: angiotensin I, AT-II: angiotensin II, ACE: angiotensin-converting enzyme, Ca: calcium, K: potassium, AC: adenylate cyclase, GC: guanylate cyclase. (G) protein G, (E) enzyme, (R) receptor (ET: endothelin, TX: thromboxane, PG: prostaglandins).

In healthy subjects, the muscarinic receptors on the endothelium are activated by acetylcholine (ACh), a neurotransmitter which sets off nitric oxide production. Platelets release substances such as adenosine diphosphate (ADP), adenosine triphosphate (ATP) and 5-hydroxytryptamine (serotonin: 5-HT) which trigger the release of NO and prostacyclin from the endothelium. Thrombin, the major enzyme of the coagulation cascade, also activates the formation of NO by endothelium. The endothelium is also stimulated by substances such as histamine, catecholamines (adrenaline, noradrenaline), substance P (neurotransmitter and neuromodulator from the central nervous system), calcium gene-related peptide. Bradykinin can also stimulate the production of EDHF by the endothelial cells [10]. Hence, when platelets and the coagulation cascade are activated, intact endothelial cells release NO which acts as a negative feed-back by causing vasodilation and thus preventing vasoconstriction, but also by inhibiting platelet activation to prevent thrombus formation.

In contrast, the production of EDCFs can be increased by vasopressin (VP) and thrombin (T) through activation of their specific endothelial receptors. In particular, the effects of ET₁ produced by endothelial cells can be amplified by the components of the renin-angiotensin system, after transformation of angiotensin I (AT-I) to angiotensin II (AT-II) by angiotensin-converting enzyme (ACE) (Fig. 2). Activation of the endothelial receptors, such as serotonin-ergic receptors, can stimulate the enzyme COX in certain blood vessels, with the production of PGH₂ and TXA₂, leading to contraction.

Wall shear stress

In addition to receptor-biochemical mechanisms, mechanical factors cause endothelium-dependent vasodilation. The blood flow exerts a physical force on the vessel wall which can be resolved into two principal vectors; 1) shear stress, which is parallel to the vessel wall and represents the friction between the flowing blood and the endothelial surface of the vessel wall, 2) tensile stress, which is perpendicular to the vessel wall and is due to the dilating force of blood pressure. The whole vessel wall, including the endothelium, smooth muscle cells and the extracellular matrix is exposed to it. In contrast, only the inner surface of the vessel wall composed of the endothelial cells, is exposed to the frictional force of shear stress [15]. This force passed to the vascular wall and moves the endothelium and subintimal layer towards the underlying layers in the direction of the blood flow, so explaining the minimal change in blood vessel diameter, related to the activation of mechanoreceptors by shear stress. There are two broad mechanisms underlying the interaction between blood flow and the endothelium [16]. One is activation of a calcium/calmodulin complex dependent receptor (*shear stress*

receptor), leading to a rapid post-translational activation of eNOS. This signal is based on potassium channels and G-protein coupling (Fig. 1) [17]. The other is the release of NO by an as-yet-unknown direct effect on the endothelial cells. This action is independent of intracellular calcium, but is probably due to phosphorylation of MAP kinases and/or tyrosine kinase activity.

Stretch force

Similar to the shear stress effect, the stretch force applied on the endothelium can modulate the production of vasoactive substances by endothelial cells, particularly vasoconstrictors factors, by activating the ionic channels-mechanoreceptor pathway (*stretch activated channels*) [18].

Hypoxia and ischemia

They can stimulate the production of NO and secretion of prostacyclins and cause endothelium-dependent vasodilation. Selective luminal hypoxia can cause a 11% dilatation in segments of femoral artery or aorta from rabbits [19]. This hypoxia-induced dilatation of intact segments is significantly inhibited by nitric oxide inhibitors. But hypoxia is associated with increases in plasma endothelin-1 or other EDCR factors, particularly at high altitudes, and can cause endothelium-dependent contraction (Fig. 1).

Other functions of the endothelium

Permeability

The process of adhesion is necessary for a variety of cell functions, including differentiation, growth, migration and the response of the cell to its external milieu. New adhesion molecules have been placed in the selectin family (particularly selectin-E), and in a superfamily of immunoglobulins, including intercellular cell adhesion molecules (ICAMs: include ICAM-1 and ICAM-2), vascular cell adhesion molecule (VCAM-1), and platelet/endothelial cell adhesion molecule (PECAM-1). ICAMs and VCAMs play an important role in the adhesion of circulating blood cells to vascular tissue, as in the inflammatory response to vascular injury. Selectins are produced by stimulated endothelial cells: they mediate the loose contacts between leucocytes and endothelial cells that allow the "tank-treading" of leucocytes over the endothelium [20]. The integrins derived from activated leucocytes interact with ICAMs and regulate leucocyte adhesion. Nuclear factor-kappa B (NF- κ B) is a transcription factor that has a pivotal role in inducing genes involved in physiological processes as well as in the response to injury and infection [21]. This factor is important in the phenotypic changes of the endothelium, as it promotes the release of proinflammatory interleukins (interleukin-1 (IL-1)) and growth factors,

activation of monocyte chemotactic protein (MCP-1), and the synthesis of adhesion proteins (VCAM-1, ICAM-1). The adhesion molecules are produced upon stimulation of endothelial cells by tumor necrosis factor- α (TNF α), and other cytokines, such as IL-1 or interferon γ . These stimulations occur in several clinical and biological conditions such as smoking, hypercholesterolemia and oxidized LDL production.

Thrombosis and hemostatic factors

Intact endothelial cells are important for the interaction between cells and the blood stream. *In vivo*, endothelial cells have pronounced antithrombotic properties. The endothelium secretes the tissue-type plasminogen activator (t-PA), a potent thrombolytic substance [22], in response to stimulation by noradrenaline, thrombin, vasopressin and stasis. The cyclooxygenase pathway may also play a role in coagulation balance by producing prostacyclin PGI₂ or thromboxane A₂, but under physiological conditions, endothelial cells have antithrombotic properties due to a favourable PGI₂/TXA₂ ratio.

However, endothelial cells can produce the prothrombotic and procoagulant von Willebrand factor (vWF), and the profibrinolytic factor plasminogen activator inhibitor-1 (PAI-1) in response to inflamma-

tion, or stimuli such as IL-1, TNF α , lipopolysaccharide, and oxidized LDL [23]. The platelet activating factor (PAF) can also be produced by endothelial cells in response to these stimulations, and this allows to platelets activation and aggregation, causing vasoconstriction.

In summary, the endothelium plays a key role in the complex relationship between the container (artery) and its contents (blood). This is why the endothelium is considered to be the "brain" of the artery.

■ HOW TO ASSESS ENDOTHELIAL FUNCTION

The function of the endothelium can be evaluated by several methods and in different sites, each of which has both advantages and disadvantages, and focuses different aspects of endothelial cell function (Table I).

In vitro direct methods

These studies are carried out on cells in culture or on isolated arteries, and the mediators are characterized in endothelial cells in culture. The principal ad-

TABLE I. Methods for exploring endothelial function.

Type	Stimulus	Parameters measured	Evaluation techniques
<i>In vitro</i> direct methods	Pharmacological (L-NAME, phenylephrine, noradrenaline, inhibitors of NOS and COX)	Flow	Cell culture
	Physiological (shear stress)	Shear rate	Isolated artery
Biochemical <i>in vivo</i> indirect methods	Pharmacological (L-arginine)	Plasma NO and PGI ₂	Biochemical assays
	Physiological (ADP, 5-HT, histamine)	Urinary nitrate, nitrites	Adhesion molecules Coagulation factors
Invasive <i>in vivo</i> direct methods	L-NMMA (intra arterial)	Arterial diameter	Ultrasonography
	Acetylcholine, serotonin, bradykinin, substance P (intra arterial)	Arterial flow	Plethysmography
Non-invasive <i>in vivo</i> direct methods	Shear stress (post-ischemic dilation)	Arterial diameter	Ultrasonography
		Arterial flow	Plethysmography Echotracking
	Dipyridamole (intra venous)	Arterial flow	Positron emission tomography

vantage of these methods is that they give a direct evaluation of the vasoactive substances secreted by the endothelium in response to changes in blood flow (pulsating, continuous) and/or to shear stress [24, 25]. Human arteries in response to acetylcholine and other endothelium-dependent dilators and inhibitors of cyclooxygenase have also been used, as well as inhibitors of eNOS and soluble guanylate cyclase. However, the response to stimuli can differ according to the type of vascular artery: for example, thrombin and adenosine cause vasodilation in renal arteries, but not in human digital arteries [26].

Biochemical *in vivo* indirect methods

These methods are not commonly used, because the biochemical system is unstable and the substances secreted by the endothelium have very short half-lives. The function of the endothelium is more often evaluated by measuring plasma NO or PGI₂. The concentration of the metabolite of nitric oxide oxidation (nitrate) is measured in the urine over 24 hours, and can be considered to be the marker of global nitric oxide secretion during this period. But the validity of this method depends on the conditions; it requires a well controlled diet and no recent trauma or infection [27, 28]. This method cannot identify the origin of the nitric oxide (endothelial, neuronal, etc), or the NOS isoform involved (inducible, endothelial) [27, 29]. Measurements of cGMP or endothelin-1 in the plasma or the urine suffer from the same limitations, and they cannot be regarded as specific markers of endothelial activity [30].

Measurements of soluble adhesion molecules (ELAM-1 now designated E-selectin, VCAM-1, ICAM-1) in circulating blood may provide an indirect index of endothelial activation in clinical and epidemiological studies [31-33].

Circulating prothrombotic and procoagulant von Willebrand factor (vWF) may reflect arterial injury [34]. However, the value of the tissue-type plasminogen activator (potent thrombolytic), as indicator of endothelial function, remains to be established.

Invasive *in vivo* direct methods

These methods evaluate the endothelial function of arteries by measuring changes in their diameter (ultrasonography) or volume (plethysmography), after cardiac catheterization to access the coronary circulation in patients with a normal coronary anatomy as well as in those with coronary artery disease. The assessment of endothelial function in healthy subjects or patients is more difficult for ethical reasons, than in experimental studies. Whatever the technique used (plethysmography, ultrasonography), drugs such as N^G-monomethyl-L-arginine (L-NMMA) and N^G-nitroarginine methyl ester (L-NAME), and/or other

neurohumoral factors (serotonin, acetylcholine, substance P, bradykinin) can be infused locally. The development of specific inhibitors of NO formation has made possible to investigate the effects of nitric oxide *in vivo*. But, we must never forget that the two families of endothelial mediators (vasoconstrictor and vasodilator) affect each other. For example, the vasoconstriction that results from inhibitors of eNOS is due not only to the withdrawal of the vasodilator substances, but also in part to the unleashing of the production of vasoconstrictor substances [35].

Infusion of L-arginine analog (L-NMMA) vasomotor response study

Infusion into an artery of N^G-monomethyl-L-arginine, a NOS-specific inhibitor of the endothelium, inhibits endothelium-dependent vasodilation [36, 37]. The resulting vasoconstriction varies inversely as the initial nitric oxide secretion. The reduction in arterial diameter after the L-NMMA infusion is considered to be a measure of satisfactory initial endothelium function. Its invasive nature and the impossibility of repeated measurements in the same patient are the principal limitations of this method [38].

Acetylcholine infusion vasomotor response studies

The technique of acetylcholine infusion was considered to be the reference method for a long time, because it specifically evaluates endothelium-dependent vasodilation. The vasodilation caused by acetylcholine is usually compared to the vasodilation produced by nitrates such as nitroglycerine or sodium nitroprusside, but the nitrates and other NO donors participate in evaluation of endothelium-independent part of arterial vasomotricity and cause vasodilation even when the endothelial function is altered. Several studies have reported that the infusion of acetylcholine causes mild vasodilation and an increase in coronary flow in healthy subjects, whereas patients with coronary artery disease have a paradoxical vasoconstriction and decreased coronary flow in large coronary arteries [39]. But the invasive nature of the test has limited its development, and the role of muscarinic receptors in the endothelium is still not well understood.

Non invasives *in vivo* direct methods

These methods can be used to estimate the vasomotor response to physical or pharmacological stimuli of the endothelium. They are based on the capacity of endothelium-dependent vasodilation to determine the quality of endothelial cells function in normal and pathological situations. The measurements made use arterial blood pressure, blood flow or blood flow velocity and vascular diameter. New methods are also being developed.

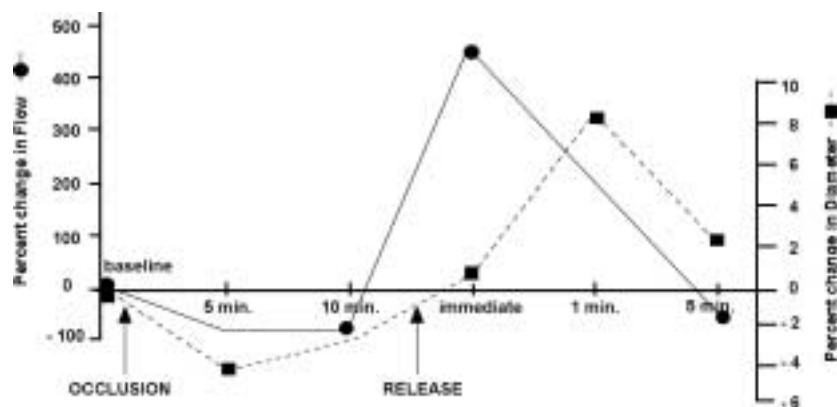


FIG. 3. Evaluation of endothelial function using high-frequency ultrasound (non-invasive technique). Percent changes in brachial artery flow and artery diameter during and after the release of arm occlusion in healthy subjects (from Corretti *et al.*, modified [40]).

Flow vasodilation

The circulation in the forearm is readily accessible for detecting changes in superficial arteries and measuring blood flow and diameter changes. The measurements can be made on the radial or brachial arteries, using standard ultrasonography, ultrasonic echotracking devices or plethysmography [40]. The main advantage of this method is its simplicity and feasibility, but the disadvantage is that it cannot be used as a model for all cardiovascular disease. Obstructive vascular lesions are very rarely encountered in this part of the circulation.

Post-ischemic dilation is a non-invasive method that consists of producing distal ischemia for 5 minutes by cuff occlusion of the radial or brachial artery [41, 42]. The endothelium secretes prostacyclin during ischemia [43], and the peripheral resistance decreases and blood flow increases during the cuff deflation (Fig. 3). Endothelium-dependent vasodilation is evaluated by the relative change in the artery diameter during the post-ischemic period [(post-ischemic diastolic diameter-baseline diastolic diameter)/baseline diastolic diameter] (Fig. 4) [44].

Anderson *et al.* have demonstrated a good correlation between the coronary vasodilation caused by acetylcholine and post-ischemic vasodilation in the forearm, suggesting that this method of evaluating endothelial function could be used to describe coronary artery dysfunction and consequently to be a *surrogate* endpoint in physiology and disease as well as in pharmacological studies [45].

Positron emission tomography

Myocardial blood flow and metabolic activity can be quantitatively assessed by positron emission tomography scanning [46]; both basal flow and hyperemic flow (usually in response to intravenous dipyridamole) can be used to calculate coronary flow reserve. Because the increase in myocardial flow is related to adenosine-induced increases and flow-mediated vasodilation, it is in part a measure of endothelial function. This technique is non-invasive and

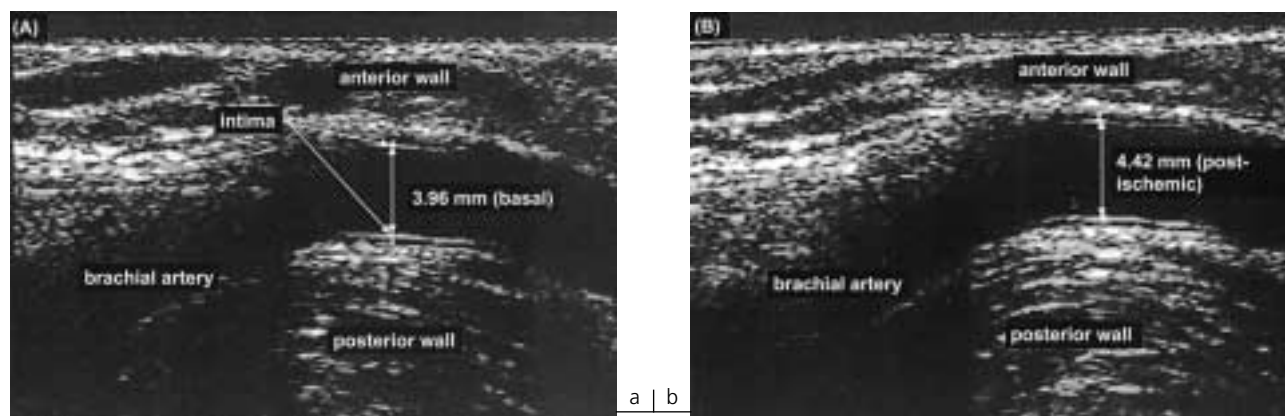


FIG. 4. Morphology of the brachial artery measured by ultrasound before and after post-ischemic dilation. Endothelium-dependent vasodilation is evaluated by the difference between the baseline diastolic diameter (A) and the post-ischemic diastolic diameter (B) of the artery (expressed as a percentage).

may be repeated in the same patient, but its cost limits its development.

Endothelium independant vasodilation assessment

Systemic infusions of sodium nitroprusside or other nitrovasodilators have been used to test specifically the sensitivity of vascular smooth muscle to nitric oxide, because these agents cause endothelium-independent vasodilation [47-49]. This approach allows the separation of the abnormalities linked to altered smooth muscle reactivity (also named endothelium-independent vasodilation response), from those of endothelial cell dysfunction. The post-ischemic dilation method can be used to evaluate the endothelium-independent vasodilation by the relative change in the artery diameter during the post-sublingual nitroglycerine period [(post-sublingual nitroglycerine diameter-baseline diastolic diameter)/baseline diastolic diameter] [44]. This evaluation can be useful in diabetes mellitus, for determining the alteration of sympathetic nervous system linked to diabetic neuropathy.

■ ENDOTHELIAL DYSFUNCTION AND THE DEVELOPMENT OF ATHEROSCLEROSIS

A satisfactory endothelium-dependent vasomotricity is an indication of the capacity of the endothelium to secrete the principal vasorelaxant factor (NO) in response to physiological or pharmacological stimuli under normal physiological conditions. However, the regulation of endothelial cells can be altered in some physiological states (age, diet, physical activity) as well as in diseases (inflammation, atherosclerosis).

Endothelial dysfunction is defined as a change towards injurious processes and it contributes to vasospasm, vasoconstriction, excessive thrombosis and abnormal vascular proliferation [50]. Endothelium vasomotricity and permability, the balance between coagulation and fibrinolysis, the composition of the subendothelial matrix, leukocyte extravasation and the proliferation of vascular smooth muscle cells can all be altered (*Table II*).

Endothelial dysfunction leads to leukocyte adhesion *in vitro*, and the infiltration of monocytes, macrophages and lipoproteins into the artery wall. This is the first stage in the formation of foam cells during atherosclerosis [51]. The decreased secretion of NO and prostacyclin by the endothelium increases platelet adhesion and the secretion of platelet growth factors. The enhanced secretion of platelet-derived growth factor (PDGF) leads to the migration of smooth muscle cells and their proliferation by growth factors (VEGF, TGF β) [3, 52-54].

Endothelial dysfunction *in vivo*, characterized by a reduction in relaxing factors and an increase in con-

TABLE II. *Properties of endothelium.*

Function	Substances and factors
Vasoregulation	
– relaxation	NO, EDHF, PGI ₂
– contraction	ET-1, PGH ₂ , TXA ₂
Permeability	
– inflammation and leukocytes extravasation	VCAM-1 and ICAM-1, PCAM-1, P and E selectin
Vasculogenesis/angiogenesis	VEGF, TGF- β
Hemostasis	
– coagulation	PGI ₂ , TXA ₂ , vWF
– fibrinolysis	t-PA, PAI-1

tracting factors, is the main feature of the initial events in the development of vascular diseases. Zeiher *et al.* reported endothelial dysfunction in the coronary circulation in high cardiovascular risk patients but without any angiographically evident atherosclerosis [55]. Several authors have demonstrated that the physiological impairment of endothelial function is linked to age [39], hypercholesterolemia [56], smoking (active and passive) [57], hypertension [58], diabetes mellitus [59, 60] and heart failure [61, 62]. The fact that the offspring of patients with essential hypertension have impaired endothelium-dependent vasodilation linked to a defect in the nitric oxide pathway, suggests that the endothelial dysfunction precedes the onset of essential hypertension [63]. Clarkson *et al.* have also found that healthy young subjects, with a family history of premature coronary disease and free of other cardiovascular risk factors, had impaired endothelium-dependent vasodilation [64]. These results suggest a genetic influence on early endothelial dysfunction. The studies of both Taddei and Clarkson raise the question of a primary defect or a consequence of endothelial dysfunction in the development of cardiovascular diseases. A recent prospective study carried out over 7.7 years, demonstrated that coronary endothelial vasodilator dysfunction predicted the long-term progression of atherosclerosis and the outcome of cardiovascular events [65]. Three tests were used to assess coronary endothelial vasoreactivity (acetylcholine-induced vasoreactivity, the cold pressor test and flow-dependent dilation); All were significantly altered in patients experiencing cardiovascular events. All the three tests were independent predictors of cardiovascular events rates in multivariate analysis.

In summary, endothelial dysfunction sequences and the mechanisms of atherosclerosis point to endothelial dysfunction as a first stage in the development of atherosclerosis [66, 67] and cardiovascular disease [68]. This is based on experimental and epidemiological data, and large interventional trials are now needed to establish how far endothelial dysfunction can or cannot predict clinical outcome. The excessive cardiovascular morbidity and mortality in diabetes mellitus has led us to examine the mechanisms underlying features of the disease in the second part of this article. We will examine the roles of hyperglycemia, insulin resistance, oxidative stress and dyslipidemia as cumulative factors involved in the endothelial dysfunction of type 2 diabetic patients. There is now considerable evidence to suggest that these factors alter early the endothelial function and accelerate the development of atherosclerosis in this population.

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