Endothelial Dysfunction and Coronary Artery Disease

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For several decades, the vascular endothelium was considered a unicellular layer acting as a semipermeable membrane between the blood and the interstitium. Recently, it has been demonstrated that the endothelium performs a large range of important biological functions, participating in several metabolic and regulatory pathways. Along with long-known specialized functions like gaseous exchange in the pulmonary circulation and phagocytosis in the hepatic and splenic circulation, the vascular endothelium performs universal roles in the circulation that include participation in thrombosis and thrombolytic control, vascular growth, platelet and leukocyte interactions with the vascular wall, and vasomotor tone.

The study of endothelium-dependent vasomotor reactivity has produced over the years, scientific evidence fundamental for the understanding of the endothelium's role in physiological and pathological situations. In 1977, Moncada et al, published the first report indicating that the endothelium plays a central role in the control of vascular tone via the production of vasoactive substances [1]. In 1980, Furchgott and Zawadzki [2] demonstrated in an experimental preparation of the rabbit aorta, the obligatory role played by endothelial cells in vascular relaxation in response to effectors like acetylcholine, and postulated the existence of a vascular relaxing factor derived from the endothelium. In 1987, two research groups, lead by Ignarro et al [3], and by Palmer et al [4], demonstrated that the relaxing factor derived from the endothelium was nitric oxide, an odorless gas until then considered as a mere pollutant.

Endothelial dysfunction was first characterized in humans in 1986 by Ludmer et al, [5] who demonstrated that atherosclerotic coronary arteries contracted in response to intracoronary infusion of acetylcholine, while normal coronaries showed dilatation. In 1992, endothelial dysfunction was documented by Celermajer et al [6] in children and otherwise healthy young adults with risk factors for atherosclerosis.

Under physiological conditions, the endothelium keeps a reduced vasomotor tone, prevents leukocyte and platelet adhesion, and inhibits the proliferation of vascular smooth muscle cells. In contrast, endothelial dysfunction appears to play a pathogenic role in the initial development of atherosclerosis [7-9] and of unstable coronary syndromes [10], being associated with atherosclerotic disease risk factors [11-18], and being present even before vascular involvement becomes evident [6,19-21].

Recent clinical studies have demonstrated that some drugs well known to reduce the incidence of cardiovascular events, improve endothelial function [22-25]. On the other hand, clinical interventions like the continuous administration of organic nitrates and percutaneous coronary interventions may be associated with adverse effects on the vascular endothelium. In the present article, we will discuss vascular endothelial function versus dysfunction, and their impact on cardiovascular disease, in particular atherosclerosis.

The endothelium in cardiovascular homeostasis - Vascular endothelium may be considered a dynamic, heterogeneous organ, having secretory, synthesizing, metabolic, and immunological functions, vital to human beings [26]. The endothelium regulates the flow of nutrient substances, of various biologically active molecules, and of blood cells through the entire human body. It is selectively permeable, possessing various cell membrane receptors for molecules that include proteins (growth factors, coagulation, and anticoagulation proteins), lipid-transporting particles (LDL), metabolites (nitric oxide, serotonin), and hormones (endothelin-1). The endothelium plays a central role in the regulation of vascular tone and blood flow by the secretion and capture of paracrine vasoactive substances, contracting or dilating specific vascular beds in response to various stimuli.

The endothelium also possesses important anticoagulant, antiplatelet, and fibrinolytic actions. Endothelial cells are the largest sites of reactions involving thrombin [27]. Some of the stimuli that activate platelets (adenosine diphosphate and thrombin) also stimulate the release of prostacyclin by the endothelium, inhibiting platelet aggregation [1,28]. In response to stimuli like noradrenaline, vasopressin, thrombin, and vascular stasis, endothelial cells secrete tissue plasminogen activator [29], a potent thrombolytic agent with wide clinical application, thus providing a defense against uncontrolled coagulation. Other hemostatic factors secreted by the endothelium, include plasminogen activating factor (PAI-1) inhibitor, von Willebrand

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factor, and thrombomodulin. When stimulated by certain physical or chemical factors, the endothelial cell undergoes phenotypical modifications that determine its transformation into a thrombogenic surface. The dynamic equilibrium existing between these two states often permits the endothelial cell to return to its basal state, once the thrombogenic stimulus has ceased.

Injury or activation in response to various pathological factors leads to modifications of the endothelial cell's regulatory functions. The endothelium becomes incapable of maintaining vascular homeostasis. This characterizes a condition of endothelial dysfunction, which can be defined as an imbalance between relaxing and contracting factors, between procoagulant and anticoagulant mediators, or between stimulants and inhibitors of cell growth and proliferation, respectively [30].

Endothelial vasomotor function - The endothelium plays a fundamental role in the regulation of vasomotor tone via the synthesis and release of vasodilator substancesnitric oxide, prostacyclin, and the endothelium-derived hyperpolarizing factor, as well as by the liberation of vasoconstrictor substances like endothelin-1 and platelet-activating factor. Nitric oxide is probably the main mediator of vasomotor tone in physiological situations, small amounts being continuously secreted by the endothelial cells [4,31] to maintain a reduced arterial tone in the systemic and pulmonary circulations [32]. The vasodilator activity of nitric oxide is due to its interaction with the iron atom of the heme prosthetic group of guanylate cyclase, causing its activation and increasing the intracellular levels of cyclic guanidine monophosphate (cGMP), [33]. In smooth muscle cells, this decreases intracellular calcium concentration, causing vascular relaxation [34].

Nitric oxide is a free radical produced by the oxidation of L-arginine to L-citrulline, via nitric oxide synthetase, an enzyme that has at least three isoforms [35]. Nitric oxide synthetase type III is a constitutive enzyme of the endothelial cell, which continuously produces small amounts of nitric oxide. In contrast to other vasomotor agents (prostacyclin, endothelin-1, and the platelet activating factor), which are synthesized primarily in response to local factors, the production of nitric oxide is regulated by various chemical and physical stimuli.

Endothelial cell constitutive nitric oxide synthetase can be activated by stimuli that include thrombin, adenosine diphosphate, bradykinin, substance P, muscarinic agonists, catecholamines, and shear stress [12]. Estrogens and shear stress stimulate the expression of this synthase's gene. Two other forms of nitric oxide synthetase are presently known: the neuronal constitutive form (type I) and the inducible form (type II). The latter has been observed in various cell types, including vascular smooth muscle, the endothelium, and macrophages. Inducible nitric oxide synthetase is activated by cytokines like interleukin-1β and the tumor-necrosing factor, being capable of producing large amounts of nitric oxide in inflammatory processes.

In the presence of a normal endothelium, the release of nitric oxide in response to catecholamines counteracts alpha-adrenergic vasoconstrictor effects. In contrast, when the endothelium is dysfunctional, an increase in coronary vasoconstriction in response to adrenergic stimuli occurs [36,37]. The increased synthesis of nitric oxide consequent to shear stress, contributes to the flow-mediated phenomenon of vasodilatation that is an important auto-regulatory physiological mechanism [38]. The production of nitric oxide can be blocked in vivo by analogues of L-arginine, like N^G–monomethyl-L-arginine (L-NMMA). Such blockade has been considerably useful for the study of the role of nitric oxide in physiological and pathological situations. The infusion of L-NMMA in the brachial human circulation leads to an increase in vascular peripheral resistance, and intravenous infusion causes an increase in systemic arterial pressure. These findings indicate that the vasculature is in a constant state of vasodilatation due to the continuous release of nitric oxide (fig. 1).

In addition to the modulation of vasomotor tone, endothelial cell-derived nitric oxide has several important vascular effects. Nitric oxide inhibits adhesion, activation, and platelet aggregation [39] and promotes platelet deaggregation, in part by a cGMP-dependent mechanism. Nitric oxide produced in response to thrombin inhibits platelets and modulates blood coagulation. Nitric oxide derived from the endothelium also inhibits leukocyte adhesion to the endothelium [40,41], migration [42], and proliferation [43] of vascular smooth muscle cells and stimulates the migration and proliferation of endothelial cells [44].

The contribution of endothelial cells to the regulation of vasomotor tone involves the production of other vasodilator compounds like prostacyclin and the endothelium-derived hyperpolarizing factor. Prostacyclin is synthesized from arachidonic acid by cyclo-oxygenase [1], being rapidly produced and released from endothelial cells [45] in response to humoral and hemodynamic factors. It interacts synergically with nitric oxide, causing vasodilatation and inhibition of platelet adhesion and aggregation [46]. The stimulation of adenyl cyclase and increased intracellular concentration of cyclic adenosine monophosphate in smooth muscle cells and platelets mediate its actions. Prostacyclin does not appear to be continuously produced by endothelial cells [47], but to be synthesized in response to specific stimuli like bradykinin, adenosine diphosphate, hypoxia, and increased shear stress.

Endothelium-derived hyperpolarizing factor, another vasodilator substance produced by the endothelium, promotes vascular smooth muscle cell relaxation by increasing cell membrane conductance of potassium [48]. This factor is also secreted in response to acetylcholine and blocked by ouabain, an inhibitor of sodium/potassium ATPase. The endothelial-derived hyperpolarizing factor has not yet been isolated, and its physiological role remains uncertain.

In contrast, endothelial cells produce the most potent

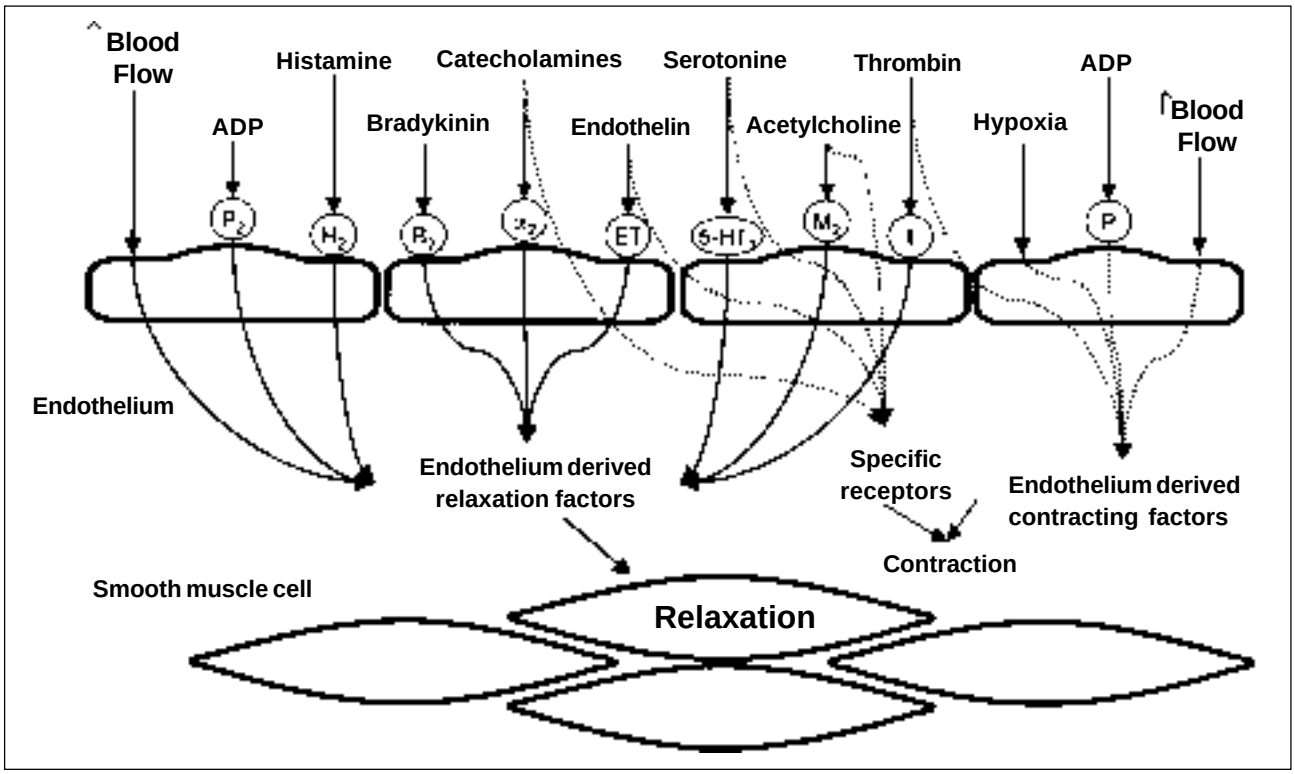

Fig. 1 – Diagram describing the action of various effectors on functionally intact endothelium. Receptor stimulation or direct action of these agents led to the liberation of endothelium-derived relaxation factors (nitric oxide, prostacyclin) that cause vascular smooth muscle cells to dilate. In contrast, serotonin, catecholamines, endothelin, acetylcholine, thrombin, hypoxia, adenosine diphosphate (ADP), and the stress of shearing (blood flow) may cause contraction of vascular smooth muscle cells. In functionally intact endothelium, vasodilatation predominates (H2- histamine receptor, a2- a-adrenergic receptor; 5-HT- serotoninergic receptor; B- bradykinin receptor; M- muscarinic receptor; P- purinergic receptor; ET- endothelin receptor; T- thrombin receptor).

vasoconstrictor known, endothelin-1 [49]. Endothelins constitute a family of polypeptides produced by various cell types. Of the three isoforms known, endothelial cells appear to produce only endothelin-1. This is a 21 amino-acid peptide formed from its inactive precursor pre-endothelin-1, which seems to exert a role as an arterial blood flow regulator in both normal and pathologic conditions [50]. In response to stimuli like thrombin, adrenalin, angiotensin II, hypoxia, and increased shear stress, endothelin-1 is released from endothelial cells, binding to specific receptors in vascular smooth muscle cells causing increased intracellular concentration of calcium leading to vasoconstriction [51]. Intramyocardial vessels are more sensitive to endothelin, suggesting that this peptide plays a major role in blood flow control. It is interesting that in functionally intact endothelia, endothelin stimulates the production of nitric oxide and of prostacyclin, which, therefore, modulates vasoconstrictor action and reduces the synthesis of endothelin itself. Two types of vascular receptors for endothelin have been identified. Receptor ETB is observed in endothelial cells, being responsible for the stimulation of nitric oxide and prostacyclin formation. Receptors ETA and ETB, observed in smooth muscle cells, mediate contraction and proliferation of these cells. A large number of endothelin receptor antagonists developed in recent years, are being tested experimentally and clinically.

Thromboxane A2 and prostaglandin H2 are constrictor factors also secreted by the endothelium. They activate the thromboxane receptor in smooth muscle cells and platelets, in opposition to the effects of nitric oxide and prostacyclin. However, the role of these substances in coronary circulation has not been clearly established. Platelet activation factor is another vasoconstrictor synthesized and released by endothelial cells in response to humoral and hemodynamic stimuli, which probably participates in the regulation of vasomotor tone. Finally, the endothelium also expresses the angiotensin-converting enzyme, which is identical to kinase II, which metabolizes bradykinin. Therefore, the angiotensin-converting enzyme also determines local levels of bradykinin, which stimulates nitric oxide and prostaglandin production. In addition, the angiotensin-converting enzyme synthesizes angiotensin, which directly stimulates the production of endothelin.

Pathophysiology of endothelial dysfunction – Endothelial dysfunction can be determined by the reduction of the endothelium-derived vasodilators, by local increases in antagonists to these substances, or by an association of these two factors (fig. 2). Reduction in the synthesis or local availability of nitric oxide have been frequently considered the major causes of endothelial dysfunction in various clinical conditions. Nitric oxide release from the endothelium is

decreased in patients with established coronary atherosclerosis [5,52]. A reduction in vascular availability of nitric oxide determines damage to endothelium-dependent vasodilatation, an increased tendency for platelet aggregation and adhesion of monocytes to the endothelium, and influences the proliferation of vascular smooth muscle cells, probably contributing to the onset and progression of atherosclerosis. In animal models of hypercholesterolemia, pharmacological inhibition of nitric oxide synthesis accelerates atherosclerosis [53], but increased availability of nitric oxide decreases and may even lead to the regression of the disease [54,55].

The inactivation of nitric oxide by oxygen-derived free radicals can be an important factor in the development of endothelial dysfunction [56]. Experimental studies suggest that antioxidant agents may reestablish endothelial function [57,58]. Vitamin C, a potent antioxidant in vivo and in vitro [59] that inhibits superoxide-mediated lipid peroxidation [60], improves endothelial function in the brachial artery of coronary artery disease patients [61], in patients with diabetes mellitus [62] and in smokers [63].

An increase in endogenous inhibitors of nitric oxide synthesis may also be involved in the genesis of endothelial dysfunction. In particular in renal insufficiency, plasma levels of methylated analogues of arginine (asymmetric dimethylarginine) are significantly increased and may compete with L-arginine in the synthesis of nitric oxide [64]. More recently, it was demonstrated that asymmetric dimethylarginine levels are increased in young individuals with hypercholesterolemia and that this increase is associated with endothelium-dependent vasomotor dysfunction [65].

Another frequently observed mechanism of vasomotor endothelial dysfunction is the increase of endothelin-1. High plasma concentrations of endothelin-1 have been reported in myocardial infarction, cardiogenic shock, unstable angina pectoris, coronary artery disease in general, cardiac failure, and essential hypertension [66,67]. Endothelin-1 action, unopposed by nitric oxide, tends to promote vasoconstriction and proliferation of vascular smooth muscle cells in states of endothelial dysfunction [68].

Evaluation of endothelial function – The most frequently employed method in clinical studies of endothelial function has been the evaluation of endothelium-dependent vasomotor responses to pharmacological stimuli or modifications of blood flow in conduction arteries and resistance vessels. In humans, the study of endothelial control of vascular tone is limited by various factors that need to be considered for adequate interpretation of results obtained. These limitations are related to the pharmacological and physical interventions used to stimulate endothelium-dependent mechanisms of vasodilatation and to the methods used to measure vascular response secondary to such interventions.

The majority of clinical studies have evaluated endothelial function in regional circulatory beds, in particular

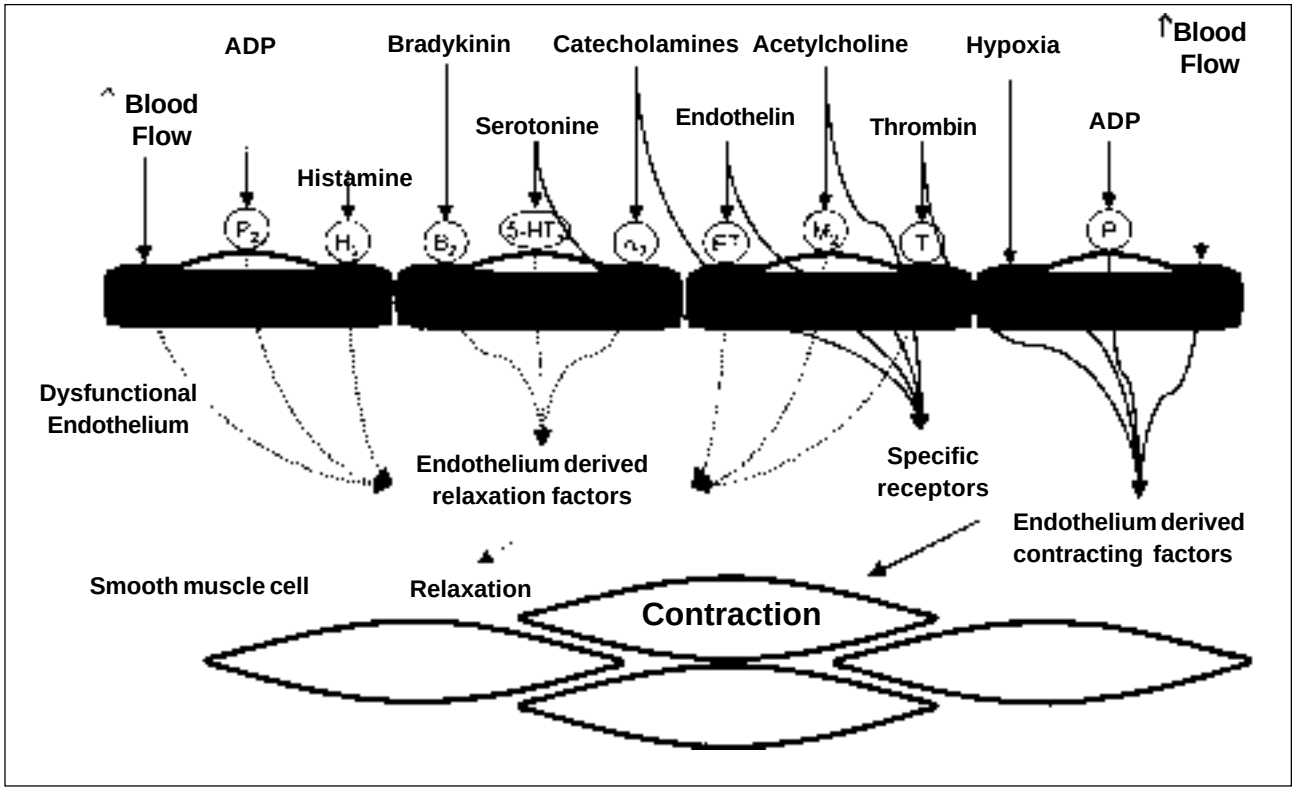

Fig. 2 – Diagram describing the actions of various effectors on dysfunctional endothelium. In the presence of endothelial dysfunction, a reduction in the action of endothelium-derived relaxation factors occurs, with predominance of vasoconstriction (H2- histamine receptor, a2- a-adrenergic receptor; 5-HT- serotoninergic receptor; B- bradykinin receptor; M- muscarinic receptor; P- purinergic receptor; ET- endothelin receptor; T- thrombin receptor).

the forearm or the coronary circulation. The administration of endothelium-dependent agents to regional circulatory compartments allows the use of relatively low doses. It is expected that this precaution will prevent the administered agent from setting off systemic reflex responses. The absence of modifications of blood pressure and of heart rate is normally used as evidence of a purely local effect. Although, undetected, small systemic effects with consequent reflex activation may occur. Also, the standardization of the concentrations administered is difficult to obtain, due to the variability of blood flow at basal conditions and in response to the administration of endothelium-dependent vasodilators [69]. Regarding acetylcholine, its in vivo concentration is also affected by the action of circulating pseudocholinesterase. Furthermore, the physiological role of these various agents has not yet been clearly defined.

Acetylcholine is the most frequently used agent in clinical studies of endothelial function. When infused into the coronary or brachial circulation of normal individuals, acetylcholine causes dose-dependent vasodilatation and increased blood flow. The vasodilatation is partly mediated by this increased blood flow, which in turn is caused by arteriolar dilatation with reduction of peripheral resistance. The direct action on endothelial cells of acetylcholine, associated with the increased flow of blood, leads to the production and release of nitric oxide, causing tone reduction and vasodilatation. In opposition, acetylcholine also causes vasoconstriction by its direct effect on muscarinic receptors of the vascular smooth muscle cells [70,71]. In the presence of endothelial dysfunction, inbalance occurs between the dilator (endothelium-mediated) and constrictor (smooth muscle cell-mediated) actions of acetylcholine, with predominance of vasoconstriction.

Other endothelium-dependent vasodilator agents used for the evaluation of endothelial function, include serotonin, bradykinin, and substance P. While bradykinin and substance P do not possess vasoconstrictor actions, causing solely endothelium-dependent vasodilatation, serotonin has a double effect, similar to that of acetylcholine, determining vasoconstriction by direct stimulation of vascular smooth muscle. Mental activity and exposure to cold can also be used for the study of endothelial vasomotor function. These stimuli are associated with the release of catecholamines, which have their vasoconstrictor action accentuated in the presence of endothelial dysfunction [36,37].

The vasomotor response to endothelium-dependent agents is frequently compared to the response to vasodilators that act independently of the endothelium, like sodium nitroprusside or nitroglycerin. These substances act by a common pathway that is the intracellular production or liberation of nitric oxide, leading to the activation of guanylate cyclase and relaxation of smooth muscle cells [72,73].

Dilatation of conducting arteries in response to increased blood flow has also been used as an indicator of endothelial function. One of the stimuli most commonly used to increase blood flow is reactive hyperemia determined by ischemia induced by temporary interruption of arterial blood flow, causing metabolic vasodilatation of the microcirculation and arterioles. Similar flow increases can be obtained by the administration of adenosine or dipyridamole, which cause arteriolar vasodilatation. Physical exercise and pacemaker-induced tachycardia can also be used to obtain increased blood flow. Pacemaker-induced tachycardia produces a lesser increase in flow, associated with metabolic vaso–dilatation. Physical exercise causes a complex physiological response, involving metabolic vasodilatation and systemic release of catecholamines. The use of the response to an increased blood flow as an index of endothelial function is validated by the experimental demonstration that flow-dependent vasodilatation of conductance arteries is determined by the release of nitric oxide from the endothelium [74-77].

The quantification of vasodilatation or vasoconstriction of the arterial conducts in response to a stimulus can be made by radiographic or ultrasonographic techniques or by plethysmography. The determination of the response of coronary conducting arteries to endothelium-dependent agents is obtained by the injection of radiological contrast media and measurement of coronary diameter by quantitative analysis of angiograms, preferably using computer assisted systems. The study of variations in coronary blood flow secondary to endothelium-dependent responses of the microcirculation demands the utilization of invasive methods like intracoronary Doppler. Vascular spasm in response to coronary catheter or Doppler guide wire may render the interpretation of these measurements difficult.

In the peripheral circulation, endothelial function can be evaluated in a noninvasive manner from the vasomotor response of the brachial artery or the forearm's microcirculation, using respectively, ultrasound or plethysmography. Responses of the peripheral microcirculation can also be evaluated noninvasively, by measuring blood flow by vascular Doppler. Few authors have used other vascular beds like the lower limb/femoral artery for the study of endothelial function.

Besides endothelium-dependent relaxation, other endothelial functions that may be investigated in human beings include the condition of the vascular renin-angiotensin system [78,79], adhesive endothelial properties related to leukocytes and platelets [80,81] and factors involved in thrombotic and fibrinolytic homeostasis [82]. In this connection, circulatory levels of endothelin, bradykinin, prostaglandins, von Willebrand factor, tissue plasminogen activator, and soluble forms of cell surface adhesion molecules (like E-selectin, ICAM-1, and VCAM-1) are potentially useful indicators of endothelial function. However, the functional role of some of these substances in human beings has not been clarified. Furthermore, the impact of different clinical conditions on the levels of these substances and on the concentration of the soluble forms of adhesion molecules, remains undetermined.

Clinical implications - The implications of endothelial dysfunction in cardiovascular disease are not fully unders-

tood. Nevertheless, there is convincing evidence that injury and dysfunction of the endothelium play a pathogenic role in the initial development of atherosclerosis [7-9] and, in a more delayed way, in unstable coronary syndromes [10]. Endothelial dysfunction has been associated with diverse risk factors for atherosclerotic disease [11], including the presence of hypercholesterolemia [12], smoking [13], arterial hypertension [14], diabetes mellitus [15], family history of premature coronary disease [16], hyperhomocysteinemia [17], and aging [18], even before vascular damage becomes evident.

Like atherosclerosis, endothelial dysfunction is evidenced earlier in the bifurcations of human coronary arteries [19]. In the presence of coronary atherosclerosis, the intensity of endothelial dysfunction is directly related to the atherosclerotic damage [5]. In primates, diet-induced development of atherosclerosis is preceded by endothelial dysfunction, and the regression of the atherosclerotic plaque is associated with the normalization of responses to acetylcholine [20]. It has also been demonstrated that endothelial dysfunction precedes the development of obstructive coronary disease in cardiac transplant patients [21]. To date, no studies are available that demonstrate whether other groups of patients with endothelial dysfunction will develop atherosclerosis.

A fundamental physiological function of the endothelium is to facilitate blood flow by providing an antithrombotic surface, which inhibits platelet adhesion and thrombi formation. As we have discussed, the injured or activated endothelial cell may either loose this anticoagulant activity or acquire pro-coagulant properties, or both. Although the role of the endothelium in pathogenesis of thrombosis *in vivo* has not been clearly documented, available evidence indicates that endothelial dysfunction is fundamental for the development of various thrombotic disturbances, in particular in acute ischemic syndromes.

It is probable that endothelial dysfunction in addition to involvement in the development of atherosclerosis and acute ischemic events, potentiates the development of myocardial ischemia even in the absence of obstructive atherosclerotic lesions by hindering an appropriate increase in blood flow in situations of stress. Up to 40% of the total coronary resistance resides in small diameter arteries (110-400mm) that are not under metabolic control [83]. These small arteries may importantly influence coronary resistance [84] and, consequently, maximal velocity of blood flow. Under physiological conditions, vasomotor tone of these small arteries is indirectly coupled with metabolic necessities by flow-mediated vasodilatation. This means that when arteriolar vasodilatation causes increased blood flow, the resulting increase in shear stress will increase nitric oxide production and dilate the small arteries [83-85], leading to an additional reduction in peripheral resistance and increased blood flow. When endothelial dysfunction is present, flow-mediated dilatation may be reduced or lost in small diameter arteries, causing subtotal increases in blood flow.

Several clinical studies have associated intracoronary infusion of endothelium-dependent vasodilators, with the development of angina pectoris in some patients with endothelial dysfunction. Recently, Hasdai et al. [86] demonstrated the presence of perfusion defects detected by ^{99m}Tc sestambi in patients with reduced coronary flow in response to intracoronary acetylcholine. However, the clinical relevance of these findings remains arguable, because in this study the radioactive drug was administered together with the infusion of acetylcholine. In another study, where we compared the vasomotor response to acetylcholine with results of effort myocardial perfusion scintillography or with dobutamine stress echography in patients free of significant coronary stenosis, we failed to find an association between the development of coronary vasoconstriction and the presence of reversible ischemia [87]. This incapacity of adequately increasing blood flow associated with endothelial dysfunction has been considered as one of the possible mechanisms of development of angina in patients with microvascular angina, or syndrome X. In this group of patients, we demonstrated that endothelium-dependent vasomotor dysfunction is present in more that 50% of cases, becoming progressively more severe with aging, but not being related to other risk factors for coronary artery disease [88].

In the same way, endothelial dysfunction appears to play a pathogenic role in various clinical situations, including systemic and pulmonary arterial hypertension, congestive heart failure, and septic shock.

Clinical interventions on endothelial function - Recent clinical studies have demonstrated improved endothelial function following the use of drugs like angiotensin-converting enzyme inhibitors [22], oral hypolipemic agents [23,24], and acetylsalicylic acid [25], known to reduce the incidence of cardiovascular events. At least part of the clinical benefits due to these therapeutic interventions are probably related to the reversal of endothelial dysfunction. These studies vouch for the role of endothelial function in the maintenance of vascular homeostasis.

The beneficial effects of acetylsalicylic acid in the evolution of atherosclerosis are well substantiated, being attributed to its antiplatelet action. Recently, the effects of acetylsalicylic acid on endothelial function were clinically evaluated in 19 patients with atherosclerosis or with risk factors for cardiovascular disease [25]. Acetylsalicylic acid improved endothelium-mediated vasodilatation in response to acetylcholine in atherosclerotic patients. This suggested that the drug might improve endothelial function by reducing a tendency towards vasoconstriction and thrombosis inhibiting in this way as well, the progress of atherosclerosis. Inhibition of angiotensin-converting enzyme with quinapril [22] and inhibition of HMG-CoA reductase with lovastatin [23,24] improved endothelial function in coronary atherosclerotic patients, this being a possible mechanism

for the reduction of adverse coronary events caused by the use of these drugs.

Reversal of endothelial dysfunction has also been obtained by the administration of antioxidant vitamins C and E in various clinical situations [61-63,89-92], estrogen replacement therapy [93], and the administration of folic acid to hyperhomocysteinemic [94] or hypercholesterolemic [95] patients. It remains open for discussion whether a relevant clinical benefit has been achieved by these interventions.

In contrast, other clinical interventions may be associated with adverse effects on the vascular endothelium. In two recently published clinical studies [96,97], we have evaluated the effects of potentially deleterious interventions on endothelium-dependent vasomotor function in coronary arteries. One of these studies demonstrated that the prolonged use of nitroglycerin leads to the development of endothelial dysfunction [96]. Fifteen patients were randomized to receive 0.6mg/hour of transdermal nitroglycerin for five days or to a control group. In comparison to the controls, greater coronary constriction in response to acetylcholine was observed in the patients who had received nitroglycerin; this response persisted for at least three hours following discontinuation of the nitroglycerin treatment (fig. 3). These findings are in agreement with those of animal experiments demonstrating that the continuous administration of organic nitrates leads to biochemical changes in the vascular wall such as increased oxidative stress [98] and increased production of endothelin-1 [99], which may evoke endothelial dysfunction. These results have clinical implications related to the development of nitrate tolerance and the potential for rebound following prolonged nitroglycerin therapy.

Percutaneous coronary angioplasty is another clinical intervention that might intensify endothelial dysfunction in atherosclerotic patients. Angioplasty of coronary stenosis determines a severe mechanical lesion of the vascular wall [100]. Although the injured endothelium appears to regenerate, endothelium-dependent vasodilatation remains altered for a long time, even following re-endothelialization [101,102]. These alterations in endothelial vasomotor function are associated with increased oxidative stress [103], which may be reversed by the administration of antioxidant vitamins [104]. In agreement with these phenomena, studies in humans have shown abnormal endothelium-dependent vasomotor function in arteries several months following coronary balloon angioplasty [105-107].

The long-term effects on endothelial function of different percutaneous coronary interventions are not known. Following a coronary intervention, the severety of the endothelial dysfunction may depend on the intensity of the injury, as well as on the specific type of the percutaneous intervention performed. The implantation of coronary endoprostheses, or stents, may cause more severe arterial injury [108,109], and a more intense inflammatory response in

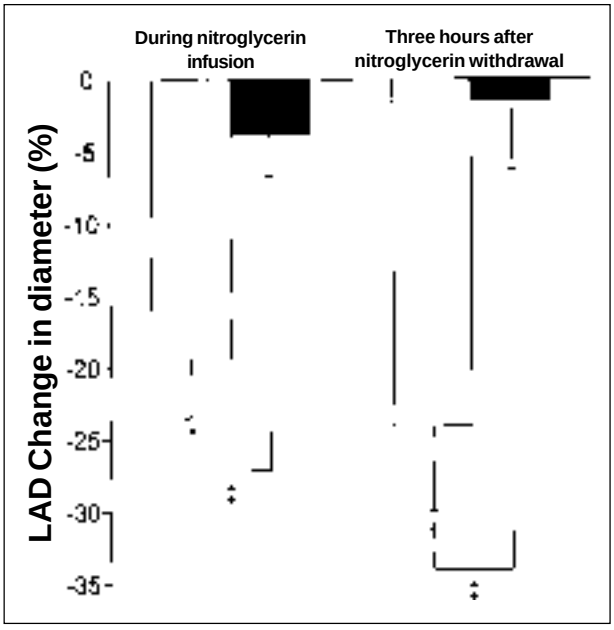

Fig. 3 – Percent modification of the average luminal diameter of the anterior descending coronary artery (LAD) from the baseline, in response to an intracoronary infusion of acetylcholine (10^{-4} molar) in patients who had received nitroglycerin versus patients in a control group. * P<0.01 versus controls during nitroglycerin therapy; † P<0.01 versus controls following withdrawal of nitroglycerin; ‡ P<0.05 in nitroglycerin group versus controls. □ = nitroglycerin group. ■ = control group.

the vascular wall than other percutaneous coronary interventions [110,111], and be associated with incomplete endothelial regeneration [112]. Recent experimental evidence indicates that stent implantation may be associated with both more severe and prolonged endothelial dysfunction [113].

To evaluate endothelial function following a percutaneous coronary intervention, we studied vasomotor responses to acetylcholine of the coronary arteries of 39 patients who had undergone more than six months earlier a percutaneous intervention for stenosis in the anterior descending artery and did not have a recurrence of the stenosis [97]. Twelve of these patients had received stents, 15 had had angioplasty by balloon catheter, and 12 had had directional atherectomy. Patients who received stents had significantly more endothelial dysfunction in comparison with those treated with balloon catheter angioplasty or directional atherectomy (fig. 4). These findings may have implications regarding the progress of atherosclerosis in coronary arteries treated with percutaneous interventions, in particular stent implantation; these findings require confirmation by additional studies.

Conclusion

The endothelium plays a central role in vascular homeostasis: endothelial dysfunction contributes to pathological conditions characterized by vasospasm, vasoconstriction, excessive thrombosis, and abnormal vascular proliferation. In fact, deterioration of endothelium–dependent vascular

relaxation has been documented in practically all forms of cardiovascular disturbances, including hypercholesterolemia, diabetes mellitus, hypertension, cardiac failure, and atherosclerosis. The vasomotor dysfunction is a reflection of a global endothelial alteration associated with the deterioration of other endothelial functions like the regulation of anti-thrombotic, profibrinolytic, leukocyte adhesive, and vascular proliferative activities. Endothelial deterioration precedes the development of atherosclerosis, becoming evident in normal individuals with risk factors for coronary artery disease. By preventing appropriately increased blood flow in stressful situations, endothelial dysfunction probably potentiates the unfolding of myocardial ischemia.

Clinical interventions using angiotensin-converting enzyme inhibitors [22], HMG-CoA reductase inhibitors [23,24], and acetylsalicylic acid [25] improve endothelial function and decrease cardiovascular events. Other interventions like the continued use of nitroglycerin and the implantation of stents appear to be associated with an abnormal response of the coronary arteries. The possibility that such therapeutic modalities cause unfavorable development of atherosclerosis and acute coronary syndromes is, at present, a speculation that requires further clinical investigation.

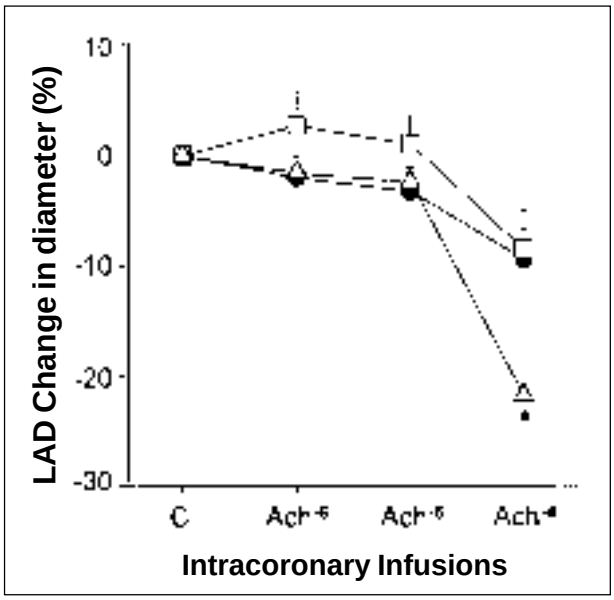

Fig. 4 - Percent modification of the average luminal diameter of the anterior descending coronary artery (LAD) from the baseline onwards, in response to an intracoronary infusion of acetylcholine (10^{-4} molar) in patients who underwent a percutaneous coronary intervention. C, Ach^{-6}, Ach^{-5}, Ach^{-4} indicate respectively, intracoronary control, and 10^{-6}, 10^{-5}, 10^{-4} molar acetylcholine (Ach) infusions. * P = 0.02 versus angioplasty by balloon catheter and directed atherectomy. —△— = stent group. —●— = angioplasty by balloon catheter group. —□— = directional atherectomy group.

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