Current perspective

Diabetic vascular disease: from endothelial dysfunction to atherosclerosis

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Cardiovascular complications are the leading cause of death in diabetes. Over the past decade a number of studies have addressed the underlying mechanisms. Derangements of endothelial function, also referred to as endothelial dysfunction, have emerged to be the crucial early step in the development of atherosclerosis and are also involved in plaque progression and clinical emergence. Endothelial dysfunction is a condition of impaired endothelium-dependent vasodilation and most important of “endothelial activation”, characterized by a proinflammatory, proliferative, and procoagulatory milieu that promotes initiation and complications of atherogenesis. A synergistic cross-talk among the conventional cardiovascular risk factors associated with diabetes contributes to disruption of endothelial integrity and accelerated atherosclerosis. This review will focus on the multifactorial nature of endothelial dysfunction in diabetes, the relationship between endothelial dysfunction, conventional cardiovascular risk factors and atherosclerosis, and the therapeutic options to improve endothelial function.

(Ital Heart J 2005; 6 (9): 703-720)

Introduction

Patients with diabetes mellitus (type 1 or type 2) have a high prevalence of cardiac morbidity and mortality with up to 75% being from cardiovascular disease\(^1\,^2\). Macroangiopathy in diabetes is manifested by accelerated atherosclerosis which affects vital organs (heart and brain) and peripheral arteries. Atherosclerosis in patients with diabetes is multifactorial and includes a very complex interaction.

During the last two decades, it has become evident that the vascular endothelium is an active paracrine, endocrine and autocrine organ that is indispensable for the regulation of vascular tone and maintenance of vascular homeostasis (Table I). Moreover, recent insights into the basic mechanisms involved in atherogenesis indicate that deleterious alterations of endothelial physiology, also referred to as endothelial dysfunction, represent a key early step in the development of atherosclerosis and are also involved in plaque progression and the occurrence of atherosclerotic complications\(^3\,^4\).

Endothelial dysfunction is characterized by a reduction of the bioavailability of vasodilators, in particular, nitric oxide (NO), whereas endothelium-derived contracting factors, particularly angiotensin II, are increased\(^5\). This imbalance leads to an impairment of endothelium-dependent vasodilation, which represents the functional characteristic of endothelial dysfunction. On the other hand, endothelial dysfunction, aside from denoting impaired endothelium-dependent vasodilation, also comprises a specific state of “endothelial activation”, which is characterized by a proinflammatory, proliferative, and procoagulatory milieu that favors all stages of atherogenesis\(^6\). All these processes can be potentiated by diabetes thus leading to accelerated atherosclerosis. The current review will discuss the role of endothelial dysfunction in the pathogenesis of diabetes-induced atherosclerosis by addressing: 1) the major molecular products elaborated by the endothelium and their roles in normal endothelial function, 2) the systemic nature of endothelial dysfunction, 3) the relationship between endothelial dysfunction, conventional cardiovascular risk factors and atherosclerosis, and 4) the therapeutic options to improve endothelial function.

Endothelial function

Endothelial cells, the major barrier between circulating blood and the vessel wall, represent an important endocrine and...
paracrine organ secreting a multitude of factors, which are involved in modulation of a number of vascular functions locally and within the circulatory bed. Regulation of vascular tone, blood fluidity and secretion of cytokines and chemoattractants in response to injury represent some of the multiple tasks of endothelial cells. Any perturbation of these delicate functions contributes to the development and progression of atherosclerosis.

**Endothelium-derived relaxing factors.** The major vasodilatory agents secreted by the endothelium include prostacyclin, NO, and the endothelium-derived hyperpolarizing factor (EDHF).

**Prostacyclin.** Prostacyclin (PGI₂) has been shown to be the predominant cyclooxygenase (COX) product in the endothelium, inhibiting platelet aggregation, causing vasodilation and preventing proliferation of vascular smooth muscle cells⁷. The individual cardiovascular effects of PGI₂ contrast those of thromboxane A₂, which causes platelet aggregation, vasoconstriction, vascular proliferation, and increased expression of leukocyte adhesion molecules⁷. Prostaglandin H₂ generated by COX from arachidonic acid⁸ is the common precursor for prostaglandins, thromboxane A₁ and PGI₂. In diabetes, nitration and inactivation of COX have been described⁹. This results in depressed formation of PGI₂ and accumulation of prostaglandin H₂ which activates thromboxane/prostaglandin H₂ receptors¹⁰. These biochemical alterations precede structural alterations in the vessel wall and are abrogated by the administration of nonselective COX inhibitors, such as indomethacin¹²,¹³. Thus, a single mechanism, depression of PGI₂ formation might result in endothelial dysfunction, increased blood pressure, accelerated atherogenesis and predispose diabetic patients to an exaggerated thrombotic response and to the rupture of an atherosclerotic plaque.

**Nitric oxide.** The endothelium-derived relaxing factor is known to be NO¹⁴, a diffusible substance, with a half-life of some seconds¹⁵. NO is derived from L-arginine in a reaction catalyzed by the enzyme NO synthase (NOS)¹⁶. Three isoforms of NOS are known to exist: endothelial NOS (eNOS), inducible or macrophage NOS (iNOS), and neuronal NOS (nNOS). NO is continually released from healthy endothelial cells, and its release is regulated by a number of physiologic stimuli (i.e. physical activity and shear stress), hormones (estrogens), and pharmacologic agents. NO produces its effects by activating soluble guanylate cyclase producing a rise in cyclic guanosine monophosphate (cGMP)¹⁷. NO produced in the endothelium diffuses to the underlying smooth muscle cells determining relaxation and vasodilation¹⁸. Other relevant biological effects of NO include inhibition of platelet activation¹⁹, limitation of smooth muscle cell proliferation²⁰, monocyte adhesion, platelet aggregation, and endothelial cell apoptosis.

**Endothelium-derived hyperpolarizing factor.** In most arterial beds a significant endothelium-dependent dilation to various stimuli persists even after inhibition...
of NOS and COX. This dilator response is preceded by an endothelium-dependent hyperpolarization of vascular smooth muscle cells which is inhibited by potassium channel blockers, mimicked by potassium channel agonists and is assumed to be mediated by an unidentified EDHF\textsuperscript{34,35}. The chemical identity of EDHF is controversial. Several chemical mediators produced by the endothelium have EDHF activity in some vascular beds, including cytochrome P-450 metabolites of arachidonic acid (epoxygenes)\textsuperscript{23}, possibly potassium\textsuperscript{24} and hydrogen peroxide\textsuperscript{25}. In diabetes, EDHF-dependent vasorelaxation is impaired\textsuperscript{26,27}. The relative contribution of NO, PGI\textsubscript{2} and EDHF in the regulation of vascular tone has not been clearly defined. However, in \textit{vitro} and in \textit{vivo} studies suggest that the contribution of NO to vascular tone is greatest in large diameter vessels, while the contribution of EDHF is greatest in small diameter vessels (i.e. mesenteric and coronary arteries) and microvessels. Also the interactions between NO, PGI\textsubscript{2} and EDHF in the regulation of vasodilation are under investigation.

**Endothelium-derived contracting factors.** Endothelium-derived contracting factors include endothelin-1 (ET\textsubscript{1}), vasoconstrictor prostanoids such as thromboxane A\textsubscript{2} and prostaglandin H\textsubscript{2} as well as components of the renin-angiotensin system (RAS).

**Endothelins.** Endothelin exists in three isoforms: endothelin -1, -2, and -3. Endothelial cells produce only ET-1\textsuperscript{28}. Translation of mRNA generates pre-pro-endothelin, which is converted to big endothelin; its conversion to the mature peptide ET-1 by endothelin-converting enzymes is necessary for the development of full vascular activity. Two isoforms of endothelin-converting enzyme have been cloned\textsuperscript{29,30}. The expression of mRNA and the release of the peptide is stimulated by thrombin, transforming growth factor-\beta (TGF-\beta), interleukin-1, epinephrine, angiotensin II, arginine vasopressin, calcium ionophore, and phorbol ester\textsuperscript{28,31}. ET-1 causes vasodilation at lower concentrations and marked and sustained contractions at higher concentrations\textsuperscript{32,33}. Two types of endothelin receptors, which bind all three endothelins, have been cloned in mammalian tissues, ETA and ETB\textsuperscript{34,35}. ETA receptors, which bind ET-1 with greater affinity than ET-3, are located on smooth muscle cells and cardiac myocytes\textsuperscript{35}. Although a blood vessel will remain constricted after ET-1 is metabolized, NO helps to restore normal intracellular calcium levels, and, thus, restore resting vascular tone\textsuperscript{36}. ET\textsubscript{B} receptors are found largely on endothelial cells, but are also present on smooth muscle cells\textsuperscript{34} and bind different subtypes of endothelins with similar affinity. ET\textsubscript{B} receptors on endothelial cells are linked to the formation of NO and PGI\textsubscript{2}\textsuperscript{34,37}. Both endothelin receptors, ETA and ET\textsubscript{B}, on vascular smooth muscle cells\textsuperscript{33-35} mediate vasconstriction and proliferation\textsuperscript{38,39}. Furthermore, ET-1 potentiates the effect of other vasoconstrictors such as serotonin and norepinephrine even at sub-threshold concentrations, which do not induce any contractile response itself\textsuperscript{40}.

The regulation of endothelin receptors is complex. Upregulation of ETA receptors is induced by epidermal and basic fibroblast growth factors, cyclic adenosine monophosphate (cAMP), and estrogen, while down-regulation results from endothelins, angiotensin II, platelet-derived growth factor, and TGF-\beta. For ET\textsubscript{B} receptors, C-type natriuretic hormone, angiotensin II, and basic fibroblast growth factor cause upregulation, and cAMP and catecholamines result in downregulation\textsuperscript{41}. The actions of endothelins in various organ systems and tissue beds are due to different expression of the two receptors and their differing affinities for the endothelin subtypes.

**Vasoconstrictor prostanoids.** Agonists such as arachidonic acid, acetylcholine, histamine and serotonin can evoke endothelium-dependent contractions that are mediated by thromboxane A\textsubscript{2} or prostaglandin H\textsubscript{2}\textsuperscript{36} products of the COX pathway. These factors activate the thromboxane receptor in vascular smooth muscle and platelets and hence counteract the effects of NO and PGI\textsubscript{2} in both cells. In addition, the COX pathway is a source of superoxide anions, potent inactivators of NO. In this light F\textsubscript{2\alpha}-isoprostanoids, a novel family of isomers of prostaglandin F\textsubscript{2\alpha}, formed from the non-enzymatic peroxidation of arachidonic acid in \textit{vivo}\textsuperscript{45}, have been shown to be elevated in type 2 diabetes mellitus. The major F\textsubscript{2\alpha}-isoprostanate formed in \textit{vivo}, 8-iso-prostaglandin F\textsubscript{2\alpha}, in addition to providing a sensitive marker of oxidant stress in diabetes\textsuperscript{46}, has recently been characterized to be a highly potent coronary vasoconstrictor per se\textsuperscript{47} as well as a stimulatory factor for endothelial ET-1 release\textsuperscript{48}.

**Angiotensin II.** The endothelium is crucially involved in the activation of the RAS since the angiotensin-converting enzyme (ACE) is located on endothelial cell membranes and transforms the biologically inactive angiotensin I into angiotensin II\textsuperscript{49}. Depending on the vascular tissue, non-ACE-peptidases may also contribute to the conversion of angiotensin I\textsuperscript{50}. In addition, ACE is also responsible for the breakdown of bradykinin, a major physiological stimulus for endothelial NO and PGI\textsubscript{2} release\textsuperscript{51,52}.

Angiotensin II acts both as a circulating and local tissue hormone as well as a neuromodulator in the central nervous system\textsuperscript{53}. While circulating angiotensin II generated by ACE located on the lung en-
endothelium is implicated in acute cardiovascular homeostasis, local vascular angiotensin II appears to provide maintenance of vascular tone, tissue function and structure in the long term. Angiotensin II evokes proliferation and migration of vascular smooth muscle cells predominantly via the angiotensin type 1 (AT1) receptor. Furthermore, RAS activation is involved in superoxide production. In experimental models of cardiovascular disease increased vascular superoxide generation impairs endothelium-dependent vascular relaxation via inactivation of NO as mentioned previously. In contrast to that observed in norepinephrine-treated rats, vascular superoxide anion production is doubled and associated with blunted endothelium-dependent relaxation to acetylcholine in rats that were made hypertensive with angiotensin II. Interestingly, the activity of NADH/NADPH oxidases, that are an important source of superoxide anions, is increased by angiotensin II both in vitro and in vivo. Even sub-threshold concentrations of angiotensin II that do not cause blood pressure elevation are shown to double NADH activity and superoxide production.

Endothelium and coagulation factors. Type 2 diabetes is a procoagulant condition. Both the coagulation and fibrinolysis cascades are dysfunctional in diabetes. Diabetic patients exhibit a pattern of coagulation factors that promote thrombosis by increasing thrombin formation or retard thrombolysis by reduced fibrinolytic potential. The prothrombotic state is characterized by increased levels of fibrinogen, which contribute to fibrin clot formation and platelet aggregation. Fibrinolytic activity has been reported to be low in type 2 diabetes. This is thought to be due to high levels of plasminogen activator inhibitor-1 (PAI-1), which inhibits the formation of fibrinolytic plasmin from plasminogen favoring both thrombosis and defective dissolution of clots once formed. These factors are important in plaque formation.

However, central to fatal events in atherosclerosis is plaque rupture and adherence of platelets. In healthy vessels, PGI2 and NO combine to prevent platelet adherence to the endothelium and platelet aggregation. These antiaggregants are released continually by healthy endothelium, but their synthesis is enhanced in the vicinity of aggregating platelets by thrombin, bradykinin and by platelet-released serotonin, platelet-derived growth factor, interleukin-1, and adenosine diphosphate. Different abnormalities in platelet function have been described in diabetic patients. Platelets in type 2 diabetic patients adhere to the vascular endothelium and aggregate more readily than those in healthy people. The molecular basis of this defect has been identified in a defective response to PGI2 and NO generated by the vascular endothelium. Since most coronary events occur with less than one third narrowing of the vessel lumen, the problem of platelet hyper-aggregability is crucial in the signal event of atherosclerosis in diabetes.

Endothelium and vascular structure. The endothelium has an important role in the regulation of vascular architecture and chronic alterations of hemodynamic stress can mediate vascular remodeling. In fact, removal of the endothelium, for instance mechanically by a balloon catheter, invariably leads to immediate deposition of platelets and white blood cells and to intimal hyperplasia at the site of injury. This suggests that the endothelium also regulates vascular structure and that its presence assures quiescence of vascular smooth muscle cells. Endothelial cells can have indirect and direct effects on vascular structure. NO and PGI2 inhibit the adhesion of platelets to the vessel wall. If at sites of endothelial dysfunction or denudation platelets do adhere to the blood vessel wall, they cause contraction and stimulate proliferation and migration of vascular smooth muscle cells. In addition, NO inhibits the adhesion of monocytes which are an important component of the atherosclerotic plaque and also source of growth factors and cytokines. Chronic inhibition of NO in rats increases wall to lumen ratio and stimulates perivascular fibrosis. The importance of NO in maintaining the vascular architecture is also confirmed by studies on eNOS knockout mice. In these animals the natural remodeling process occurring after a reduction in arterial blood flow is absent. Another main endothelial product which has striking effects in this setting is ET-1 which is mitogenic and activates protooncogene expression in vascular smooth muscle cells. An important regulator of the vascular structure is also angiotensin II. In endothelial cells and vascular smooth muscle cells, angiotensin II stimulates the expression of adhesion molecules, such as intracellular adhesion molecule-1 and the vascular cell adhesion molecule, which enhance the adhesion of circulating monocytes to endothelial surface. In addition, angiotensin II stimulates expression of monocyte chemoattractant protein-1 (MCP-1), which promotes movement of monocytes into the vessel wall and stimulates movement of monocytes. Moreover, angiotensin II stimulates platelet aggregation and thrombosis as well, the latter by transactional activation of PAI-1 gene expression. Angiotensin II also promotes migration and growth of the vascular smooth muscle cells, along with increased expression of TGF-β, and is a major regulator of vascular remodeling. Under physiological conditions potent inhibitors of vascular smooth muscle migration and proliferation, such as NO, glycosaminoglycans and TGF-β, prevail. This may explain why the blood vessel wall normally is in a quiescent state and does not exhibit proliferative responses. At variance, in certain pathological conditions, endothelial cells produce various growth factors which may contribute to proliferative responses.
Endothelial dysfunction in diabetes

Definition and pathogenesis. Endothelial dysfunction is characterized by defective endothelium-dependent vasorelaxation, which precedes structural changes of the vessel wall. Endothelial dysfunction is strongly associated with atherosclerosis, as demonstrated by the fact that many clinical studies have reported that endothelial dysfunction, both in the coronary and in the peripheral districts, represents an independent predictor of future cardiovascular events.

Endothelial dysfunction has been demonstrated in a number of animal models of diabetes, and, most important, in diabetic patients in different vascular beds. Hyperglycemia is the necessary condition for the development of endothelial dysfunction. Healthy volunteers undergoing hyperglycemic clamp demonstrate evidence of endothelial dysfunction suggesting that short-term hyperglycemia per se has adverse effects on vascular function. The underlying mechanisms have been widely addressed. Crucial to the pathogenesis of endothelial dysfunction in the early stages, in the absence of structural changes of the vessel wall, is reduced NO bioavailability. Strategies aiming at increasing NO bioavailability, i.e. by overexpressing eNOS, result in restoration of endothelial function in animal models of diabetes. A number of factors potentially involved in quenching NO have been identified in diabetes, including overproduction of oxygen-derived free radicals, increased circulating levels of asymmetric dimethylarginine, an irreversible inhibitor of NOS, activation of protein kinase C (PKC), and production of advanced-glycation end-products (AGEs). Oxygen-derived free radicals are especially felt to play an important role in diabetic vascular disease. There is considerable evidence that generation of oxygen-derived free radicals, especially superoxide anion, is increased in diabetes. Endothelial cells exposed to high glucose have increased superoxide generation and disturbed cell proliferation, which is restored by overexpression of endogenous antioxidant enzymes, suggesting a role of free radicals in this process. In addition, overexpression of the antioxidant enzyme superoxide dismutase normalizes endothelial dysfunction in the aorta of diabetic animals. Oxygen-derived free radicals may result in impaired endothelium-dependent vasorelaxation due to increased degradation of NO. NO is rapidly destroyed by superoxide-generating systems such as NAD(P)H oxidase and is protected by superoxide dismutase. Furthermore, NO may combine with excessive quantities of superoxide to form peroxynitrite, a potent oxidant substance. Thus, increased oxidative stress may decrease the amount of available NO. The diabetic patient may be especially prone to oxygen free radical-induced damage as a deficiency of antioxidant defense mechanisms including superoxide dismutase has been reported. In diabetes, glucose-induced accumulation of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NOS, has been described. ADMA could contribute to reduce NO production and thus to endothelial dysfunction and late atherosclerosis in diabetes. Another important mediator of diabetic vascular disease is activation of PKC. In the presence of hyperglycemia, increased diacylglycerol levels activate PKC, which in turn stimulates dysfunctional eNOS activity and NAD(P)H oxidase enzyme, contributing to increased superoxide production and oxidative stress. PKC activity is increased in the aorta of diabetic animals as well as in cultured vascular cells or tissues exposed to high glucose. Selective inhibition of PKC prevents high-glucose-induced endothelial dysfunction both in vivo and in vitro. While acute hyperglycemia impairs endothelial function mainly by elevating superoxide production, chronic hyperglycemia has additional detrimental effects on the vessel wall. This condition results in accumulation of AGEs. The role of AGEs in diabetic vascular dysfunction has been extensively examined. AGEs are known to quench NO both in vivo and in vitro, and this is associated with defective endothelium-dependent vasodilation. Thus, decreased NO delivery to underlying smooth muscle cells due to accumulation of AGEs may contribute to endothelial dysfunction in diabetes. In addition, AGEs by binding with their cellular receptors, RAGE and AGE-R3, may activate a number of growth factors and inflammatory signaling pathways in endothelial and smooth muscle cells with important consequences on atherosclerosis initiation and progression. Blockade of RAGE suppresses accelerated plaque formation in animal models of diabetes. Our group has also shown that treatment with the AGE cross-link breaker ALT-711, or the inhibitor of AGE formation aminoguanidine demonstrated the ability to reduce vascular AGE accumulation in addition to attenuating atherosclerosis in diabetic apolipoprotein E-deficient mice.

Recently, a unifying hypothesis explaining activation of the major pathways involved in diabetic macrovascular disease has been developed. The central event in hyperglycemia-mediated endothelial dysfunction has been identified in enhanced superoxide production in endothelial cells deriving from increased pyruvate flux to the Krebs cycle and the mitochondrial respiratory chain. Superoxide anion, in turn, is responsible for activation of the main pathways involved in the development of diabetic vascular disease and progression from endothelial dysfunction to overt atherosclerosis (activation of PKC and increased AGE formation). Thus, the pathogenesis of diabetes-induced vasomotor dysfunction is likely to be complex; in the early stages increased generation of oxygen free radicals and decreased availability of NO are crucial to the development of endothelial dysfunction; in contrast, in the late stages accumulation of AGEs with local inflammation and remodeling of the vessel wall.
play a central role in the initiation and progression of atherosclerotic lesions.

**Risk factors of endothelial dysfunction and atherosclerosis.** Hyperglycemia is the necessary condition for the development of endothelial dysfunction and atherosclerosis, as demonstrated by the observation that type 1 diabetic patients who underwent intensive glucose-lowering therapy have decreased progression of intima-media thickness in the carotid arteries\(^\text{117,118}\). Strict glucose control alone however does not result in improved cardiovascular outcome in diabetic patients. In 1993, the Diabetes Control and Complications Trial (DCCT) Research Group did not report that intensive treatment of hyperglycemia was sufficient to significantly reduce excess risk for macrovascular disease in type 1 diabetic patients\(^\text{117}\). Similarly, in the UK Prospective Diabetes Study (UKPDS) intensive glycemic control with insulin or a sulphonylurea in type 2 diabetes resulted in a non-significant reduction in the risk of myocardial infarction and no reduction in the risk of stroke, as compared with conventional therapy\(^\text{119}\). The fact that type 2 diabetes is a multifactorial disease implicates that a number of factors, other than glucose, contribute to accelerated atherosclerosis and cardiovascular disease.

The Multiple Risk Factor Intervention Trial (MRFIT) has clearly shown that systolic hypertension, elevated cholesterol and cigarette smoking were independent predictors of mortality and that the presence of more than one of these risk factors had a greater impact on increasing cardiovascular mortality in diabetic than in non-diabetic subjects\(^\text{120}\). In addition, other risk factors have been described in diabetic patients, including obesity and diabetic dyslipidemia, characterized by increased low-density lipoprotein (LDL) cholesterol, low high-density lipoprotein (HDL) cholesterol, and high triglycerides.

As a result, it has been clearly established that a multifactorial intervention aiming at controlling the risk factors associated with diabetes results in reduced cardiovascular morbidity and mortality in these patients. The American Diabetes Association recommends not only good glycemic control but also identification and aggressive treatment of associated cardiovascular risk factors, with more stringent target levels for lipids and blood pressure than those recommended for the general population\(^\text{121}\). The most effective treatments to prevent major cardiovascular events among diabetic patients include lifestyle and pharmacologic interventions intended to treat all cardiovascular risk factors associated with type 2 diabetes in the insulin resistance syndrome, i.e. maintain glycylated hemoglobin < 7%, blood pressure < 130/80 mmHg, LDL cholesterol levels < 100 mg/dl, HDL cholesterol > 40 mg/dl, and triglycerides < 150 mg/dl. Such long-term, multifactorial intervention has been shown to reduce the risk not only of cardiovascular, but also of microvascular events by about 50%\(^\text{122}\).

**Endothelial dysfunction and atherosclerosis.** Diabetes is an independent risk factor for the development of vascular disease which in diabetic subjects is a diffuse accelerated atherosclerotic process, with an early onset\(^\text{123}\). In addition to enhancing the initiation of atherogenesis, diabetes promotes plaque instability and clinical sequela\(^\text{123}\). Furthermore coronary heart disease (CHD) in diabetic patients has an increased incidence as well as an earlier onset with clinical presentation in younger age groups and amongst female patients\(^\text{124,125}\). Atherosclerotic lesions are almost invariably present in patients who have had diabetes for 5 to 10 years or longer, and increase with age and duration of the diabetes. Moreover atherosclerotic lesions tend to develop 10 to 12 years earlier in diabetic than in non-diabetic patients\(^\text{124,126}\) and the risk of CHD is also substantially increased in patients with asymptomatic hyperglycemia\(^\text{127}\). Furthermore, myocardial ischemia due to CHD commonly occurs without symptoms in patients with diabetes\(^\text{128}\). As a result, multivessel atherosclerosis often is present before ischemic symptoms occur and before treatment is instituted. Delayed recognition of various forms of CHD undoubtedly worsens the prognosis in survival for many diabetic patients\(^\text{129}\). Hence the need for primary prevention of CHD events in diabetic subjects is paramount to their survival.

Not much is known about plaque differences in subjects with and without diabetes. Indeed, and it is yet to be clarified if the atherosclerotic lesion in diabetic patients has the same pathologic features as in non-diabetic subjects. The structural changes begin with the fatty streak and progress to the denuded ulcerated plaque, which might then rupture to occlude and cause catastrophic infarction\(^\text{130}\). This process begins in the first decade of life and precedes the development of hyperglycemia by 40 years. Initial growth of the atheromatous plaque is by lipid accumulation in the subintimal space, followed by smooth muscle cell proliferation and denudation of the endothelium. Healing occurs by formation of the fibrous plaque. If this plaque becomes unstable, it is susceptible to the forces of shear stress, tears, or ruptures, and the eroded plaque is the nidus for clot formation, which is the basis of the acute vascular event\(^\text{130}\). In atherectomy specimens, the cell-rich and necrotic areas are increased in de novo lesions in persons with diabetes\(^\text{131}\). In restenotic areas, an increased content of collagen has been found in vessels from subjects with diabetes\(^\text{132}\). In a series of coronary arteries examined after sudden death, the extent of the necrotic core of plaques, calcification, and healed ruptures were increased in patients with type 2 diabetes\(^\text{133}\). However, in type 1 diabetes, an increased content of fibrous tissue and reduced number of foam cells in plaques may provide relative stability to atherosclerotic lesions\(^\text{134}\). Recently, evidence has been obtained to support an increased content of macrophages in the atherosclerotic lesions of diabetic subjects\(^\text{135}\). This is like-
ly a consequence of an increase in recruitment of macrophages into the vessel wall due to the higher levels of cytokines present in diabetes. Our group has recently demonstrated in an experimental model of type 1 diabetes that in long-term diabetes there is not only an acceleration in the development of atherosclerosis but also an increase in the complexity of the atherosclerotic plaques characterized by increased macrophage infiltration, cellular proliferation, smooth muscle cell migration, and collagen content. Currently it is still controversial as to whether there is a difference in plaque pathology between type 1 and 2 diabetic subjects, with type 1 diabetic patients having more fibrous and calcified atherosclerotic lesions and type 2 diabetic patients having more cellular and lipid-containing lesions.

The pathogenesis of the accelerated atherosclerosis as observed in the diabetic context is still incompletely understood. As mentioned previously, there is an increased prevalence of other cardiac risk factors such as hypertension, dyslipidemia and obesity among diabetic patients. Nevertheless, epidemiologic studies suggest that these risk factors although more prevalent in diabetes, do not fully account for the increased risk of cardiovascular disease. Furthermore, although hyperglycemia, the primary clinical manifestation of diabetes, is considered to contribute to accelerated development of atherosclerosis, the DCCT research studies and more recently the UKPDS did not detect statistically significant effects of intensified glycemic control on macrovascular disease, as previously mentioned. Therefore, the increased atherosclerotic disease burden in diabetic patients raises the likelihood that factors specific to diabetes other than hyperglycemia are contributing to the increased cardiovascular disease mortality and morbidity. Several clinical and experimental observations suggest that the pathogenesis of atherosclerosis in diabetes is complex and multifactorial. Five general areas of pathogenetic mechanisms have recently been identified by the American Heart Association (Fig. 1) including metabolic derangements such as hyperglycemia, increased levels of free fatty acids, and lipoprotein abnormalities, excessive oxidation/glucoxidation leading to AGE accumulation, a prothrombotic state and endothelial dysfunction.

Endothelial dysfunction is currently thought to be not only a marker of vascular disease, but also to play an important role in its initiation, progression, and clinical emergence. Endothelial injury is now regarded as an important initial event in atherogenesis. Disruption of the functional integrity of the vascular endothelium plays an integral role in all stages of atherogenesis ranging from lesion initiation to plaque rupture. In the milieu of cardiovascular risk factors that disturb vascular homeostasis, the endothelium becomes dysfunctional resulting in enhanced production of cytokines and expression of cellular adhesion molecules by the endothelium. Adhesion molecules play a crucial role in the interaction of the endothelial surface with circulating leukocytes and mediate the recruitment of leukocytes and their accumulation in the intima of the vessel wall. Inflammation plays an important role in atherosclerosis. By promoting inflammation within the vessel wall, dysfunctional endothelium sets the stage for both initiation and progression of atherosclerotic lesions. Localized inflammation also contributes decisively to plaque instability and rupture predisposing to acute clinical syndromes. The vulnerable plaque typically contains a prominent accumulation of inflammatory cells including macrophages and T lymphocytes.

![Figure 1](https://example.com/image1.png)

**Figure 1.** In diabetes, hyperglycemia induces endothelial dysfunction which is characterized by vasoconstriction, inflammation, cellular growth, and thrombosis. By losing its protective properties, dysfunctional endothelium is a major promoter of atherogenesis and, consequently, cardiovascular events. PAI-1 = plasminogen activator inhibitor-1; TPA = tissue plasminogen activator; VSMC = vascular smooth muscle cell.
Macrophages can weaken the connective tissue framework of the fibrous cap of atheromatous plaques by secreting extracellular matrix degrading enzymes and thereby facilitate plaque rupture. In > 60% of cases, plaque disruption occurs at the plaque’s shoulder regions where the fibrous cap is thinnest and the mechanical stresses are greatest\(^\text{145}\). These shoulder regions are sites of predilection for active inflammation characterized by both activation of endothelial cells\(^\text{148,149}\) and macrophage infiltration\(^\text{145}\). Recently we have demonstrated an important role for the local RAS in activating the inflammatory process in diabetic vessels\(^\text{136}\). In fact, we have shown activation of the local RAS within the aorta in diabetic apolipoprotein E knockout mice, predominantly within the plaques and the medial layer\(^\text{136}\) associated with a significant increase in the development and progression of diabetes-induced atherosclerosis. Blockade of the RAS with an ACE-inhibitor or an AT\(_1\) receptor antagonist prevents atherosclerosis by mechanisms involving inhibition of proinflammatory molecules such as vascular cell adhesion molecule and MCP-1 and proclerotic and proproliferative cytokines such as connective tissue growth factor and platelet-derived growth factor.

Proinflammatory actions of dysfunctional endothelium potentially play an important role in converting stable atheromatous plaques in unstable plaques prone to rupture. The functional state of the endothelium may also affect the consequences of plaque rupture. If plaque rupture occurs, prevention of superimposed thrombus formation, a critical step underlying acute coronary syndromes, depends on successful inhibition of platelet aggregation and coagulation and on activation of fibrinolysis. As previously underlined NO is an important mediator in these defense mechanisms. Reduced NO bioavailability due to endothelial dysfunction may therefore facilitate thrombus formation. Dysfunctional endothelium also leads to increased production of PAI-1, an inhibitor of fibrinolysis, and reduced production of the fibrinolytic component tissue plasminogen activator contributing to a thrombogenic state.

By losing its protective properties and allowing the unopposed action of atherogenic factors on the vessel wall, dysfunctional endothelium is a major promoter of both atherogenesis and thrombosis and, consequently, cardiovascular events.

**Therapeutic options**

Restoration of normal endothelial function may favorably affect the prognosis of diabetic patients. Endothelial dysfunction is a reversible disorder, and strategies aimed at reducing cardiovascular risk factors, such as cholesterol lowering\(^\text{150}\), antihypertensive therapy\(^\text{151}\), smoking cessation\(^\text{152}\), estrogen replacement therapy in postmenopausal women\(^\text{153}\), antioxidants\(^\text{154}\), and physical exercise\(^\text{155}\), also translate into an improvement in endothelial health. Moreover, the observation that several pharmacologic interventions that improve endothelial function are associated with a decrease in cardiovascular events independent of risk factor modifications supports the concept that cardiovascular risk factors share a common pathway that leads to endothelial dysfunction, such as oxidative stress.

In addition there are other promising approaches such as the insulin sensitizers\(^\text{156}\) that may improve endothelial function and reduce progression to atherosclerosis in insulin resistance and diabetes.

**Angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists.** The RAS is likely to contribute to endothelial dysfunction, suggesting that drugs which block this pathway may be of benefit. Treatment with an ACE-inhibitor or angiotensin II receptor blocker for 4 weeks increases NO bioavailability and improves endothelial function by increasing the activity of superoxide dismutase activity\(^\text{157}\). Some studies suggest that ACE-inhibitors upregulate eNOS protein expression. Although the mechanisms are not completely understood, recent data suggest that increased formation of cAMP through PGI\(_2\) may be involved in the upregulation of eNOS with ACE-inhibition\(^\text{158}\). Results from several experimental models indicate that ACE-inhibitors improve endothelial function. In rats, long-term ramipril administration attenuates aortic nitrate tolerance\(^\text{159}\) and toxic effects on the aorta from oxidized LDL\(^\text{160}\). In addition as previously mentioned we demonstrated that treatment with the ACE-inhibitor perindopril or the AT\(_1\) receptor antagonist irbesartan prevents the development of atherosclerosis in diabetic apolipoprotein E-deficient mice\(^\text{136,161}\).

ACE-inhibitors improve endothelial function in patients with coronary artery disease\(^\text{162}\) and type 2 diabetes\(^\text{163}\). The Trial on Reversing Endothelial Dysfunction (TREND)\(^\text{162}\) showed convincing evidence for an effect of ACE-inhibition on the endothelium-dependent vasodilation in subjects with CHD. Moreover, O’Driscoll et al.\(^\text{163}\) have observed that ACE-inhibition with enalapril improved both basal and stimulated NO-dependent endothelial function in normotensive patients with type 2 diabetes. Furthermore, the recent BANFF study\(^\text{164}\) compared the effect on endothelium dysfunction in 80 patients treated with quinapril, enalapril, losartan and amlodipine. In this crossover study, quinapril was associated with a significant improvement in flow-mediated dilation. Large-scale clinical trials, such as the Heart Outcomes Prevention Evaluation (HOPE) study and the UKPDS\(^\text{165,166}\), showed that ACE-inhibitors prevent or retard the development of diabetic macrovascular complications, thus significantly reducing cardiovascular mortality and morbidity in diabetic patients.

Few data are available for the role of angiotensin receptor antagonists in improving endothelial function. Improvement in endothelial dysfunction has been
demonstrated in patients with type 2 diabetes treated with the AT₁ receptor antagonist, losartan167. In addition, AT₁ receptor blockers have been shown to exert an anti-inflammatory effect. Valsartan suppresses reactive oxygen species generation, nuclear factor-κB (NF-κB) binding, increases inhibitor kappa B expression and reduces C-reactive protein levels168. Candesartan induces a reduction in plasma concentrations of proinflammatory cytokines169. Moreover, Cipollone et al.170 have recently demonstrated that treatment with the AT₁ receptor blocker irbesartan decreases inflammation and inhibits prostaglandin E₂-dependent synthase expression in plaque macrophages contributing to plaque stabilization by inhibition of metalloproteinase-induced plaque rupture. Clinical trials have shown that AT₁ receptor blockers reduce cardiovascular mortality and morbidity, particularly in patients with diabetes171,172.

**Antiplatelet drugs.** Platelet aggregation and thrombus formation play a critical role in the initiation and development of key complications of acute coronary syndromes. Antiplatelet and antithrombotic therapies have been demonstrated to favorably modify clinical outcomes.

Aspirin is known to irreversibly inhibit COX activity suppressing platelet thromboxane A₂ production and thus aggregability173. Prevention of atherothrombotic complications constitutes the best evidence that the antithrombotic effect of aspirin is largely due to the suppression of platelet thromboxane A₂ production. Beyond the effects on platelets, aspirin has potent anti-inflammatory properties, which are prominent on the vascular endothelium as demonstrated by its efficacy in blunting vascular inflammation174 and by the observation that its protective effect in reducing cardiovascular events is prominent in individuals with elevated C-reactive protein levels175. In addition, aspirin has been shown to attenuate progression of intima-media thickness in patients with type 2 diabetes and early-stage carotid atherosclerosis176. Although its effects on endothelial dysfunction in diabetes are not clear177,178, the benefits of aspirin in both primary and secondary prevention of cardiovascular disease have been convincingly documented. Aspirin therapy is associated with a 15% reduction in major cardiovascular events in diabetic patients179. It has been estimated that 38 ± 12 major cardiovascular events per 1000 diabetic patients would be prevented if they received low-dose (75-162 mg) aspirin as a secondary prevention strategy180. Although it seems that low-dose aspirin has much smaller effects on primary prevention of cardiovascular events in diabetic patients as opposed to the general population181,182, the American Diabetes Association recommends use of low-dose (75-162 mg) aspirin both as primary and secondary prevention strategy in patients > 40 years of age or who have additional cardiovascular risk factors. In selected high-risk diabetic patients with manifested atherosclerotic vascular disease, use of clopidogrel 75 mg may result in a better outcome rather than the use of aspirin183.

**Statins and fibrates.** 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase or statins are the most effective class of drugs to reduce serum cholesterol levels. Aggressive treatment of dyslipidemia is a goal of multifactorial therapy of type 2 diabetes to reduce cardiovascular risk. Diabetic patients are considered a high-risk group and LDL cholesterol target in this population is < 100 mg/dl, with HDL cholesterol > 40 mg/dl in males and > 50 mg/dl in females and triglycerides < 150 mg/dl. In addition to the lipid-lowering effects, a number of non-lipid-related effects, the so-called “pleiotropic” effects, have recently been described for these drugs, accounting for a 30% relative risk reduction of major coronary effects184. They include upregulation of eNOS expression and NO production, downregulation of oxidative stress, anti-inflammatory actions185, favorable effects on thrombosis186 and plaque stability and reduced smooth muscle cell activation, proliferation and migration186.

Clinical trials have shown that these agents reduce cardiovascular and cerebrovascular disease in diabetic patients up to the order of 25%187,188. Effects on endothelial function are controversial. While some studies have reported ameliorated endothelial function in patients with diabetes following statin therapy, other studies have not (Table II)189-196. One possible explanation for these divergent findings is that the positive effects of statins on endothelial function are offset by the presence of hyperglycemia. Thus again, a combined therapy aiming at the correction of all of the cardiovascular risk factors associated with diabetes may result in reduced cardiovascular morbidity and mortality in this disease.

In addition to statins also the fibric acid derivatives have been used in the lipid management in diabetic patients. Fibrates, which increase HDL cholesterol values and lower triglyceride levels, activate peroxisome proliferator-activated receptor-α (PPARα) in the vessel wall197. PPARα activation decreases vascular inflammation, growth, and oxidation, and it appears to be vascular protective198. In two studies using the fibric acid derivative gemfibrozil, a reduction in cardiovascular endpoints has also been achieved199,200.

**Thiazolidinediones.** The thiazolidinediones are a novel class of insulin sensitizing drugs, which act through the nuclear transcription factor receptor PPARγ. Thiazolidinediones, ligands to the PPARγ, improve insulin-mediated glucose uptake and reverse nearly all of the components of the metabolic syndrome201. These ligands enhance expression of genes involved in the insulin-signaling cascade (primarily in the phosphatidylinositol 3-kinase pathway, which likely involves direct effects in skeletal muscle) and modulate the complicated relation between adipose tissue and skeletal muscle202. In contrast, these agents inhibit several nuclear
events activated by the mitogen-activated protein kinase pathway and, thus, decrease cell growth and movement in the vasculature and decrease circulating PAI-1 levels. Ligands to PPARγ appear to restore the balance between the two major cell-signaling pathways that are activated by insulin. It is interesting in this light that insulin sensitizers of the thiazolidinedione class have also been shown to be anti-inflammatory and potentially antiatherogenic203,204. Thus, thiazolidinediones exert a suppressive effect on reactive oxygen species generation, NADPH oxidase expression, intranuclear NF-κB binding, a stimulatory effect on inhibitor kappa B expression along with a reduction in plasma concentrations of intracellular adhesion molecule-1, MCP-1 chemokine, and PAI-1. Thiazolidinediones also suppress the plasma concentrations of tumor necrosis factor and C-reactive protein.

The first thiazolidinedione, troglitazone, was approved as a glucose-lowering therapy for patients in the United States with type 2 diabetes. Troglitazone was subsequently withdrawn from the market because of hepatotoxicity. The two currently available PPARγ agonists are rosiglitazone and pioglitazone. They are currently approved in most countries including Italy for the treatment of hyperglycemia in patients with type 2 diabetes, either as monotherapy or in combination with sulfonylureas or metformin. In the United States, both drugs have also been approved for use in combination with insulin, provided certain precautions are followed.

Troglitazone and rosiglitazone have been shown to improve endothelial function when measured in peripheral arteries204,205. Rosiglitazone has recently been shown to improve coronary artery endothelial cell function in insulin-resistant subjects when measured by positron emission tomography206. Treatment with rosiglitazone has been shown to affect endothelial function by improving vascular reactivity and markers of endothelial function204,206. In addition, several clinical studies have demonstrated that treatment with rosiglitazone has other beneficial effects on cardiovascular risk factors and markers associated with type 2 diabetes, such as reducing C-reactive protein and improving markers of plaque stability204,211-213.

Two double-blind, placebo-controlled studies have examined the effects of troglitazone on endothelium-dependent and -independent vasodilation in humans. One study showed that 8 weeks of troglitazone therapy had no effect on vascular function in patients with obesity214, whereas the other showed improvements in flow-mediated vasodilation in a subgroup of patients with type 2 diabetes newly diagnosed215. A placebo-controlled study showed reduced progression of the intima-media thickness of the common carotid artery in patients with type 2 diabetes who were treated with rosiglitazone216. There are no data on the effects of thiazolidinediones on cardiovascular events in patients with insulin-resistant conditions. In conclusion, thiazolidinediones represent a promising approach that im-

| Table II. Studies on the effects of statins on endothelial function in diabetes. |
|-----------------------------------------------|-----------------|-----------|-----------|
| Statin                                        | LDL-cholesterol (mmol/l) | HbA1c (%) | Follow-up (weeks) | Results |
| T/window et al.189                           | 80               | Atevastatin 10-20 mg/die | 4.37 ± 0.71 | 2.28 ± 0.49 | 7.9 ± 1.1 | 24 |
| Economides et al.190                         | 77               | Atevastatin 10 mg/die    | 3.9 ± 0.18  | 3.72 ± 0.23 | 7.3 ± 1.5 | 12 |
| Sheu et al.191                               | 21               | Simvastatin 10 mg/die    | 4.2 ± 0.23  |
| van Venrooij et al.193                        | 133              | Atevastatin 10-80 mg/die | 3.1 ± 0.58  |
| Tsunekawa et al.195                          | 27               | Cerivastatin 10 mg/die   | 4.37 ± 0.71 |
| Mullen et al.196                             | 84               | Atevastatin 40 mg/die    | 3.08 ± 0.92 |

↑ = FDD, plasma NOX and cGMP; ↓ = flow-dependent vasodilation; HbA1c = glycated hemoglobin; LDL = low-density lipoprotein; NOX = nitrites and nitrates; DM = diabetes mellitus; TNF-α = tumor necrosis factor-α.

cGMP = cyclic guanosine monophosphate; CRP = C-reactive protein; FDD = flow-dependent vasodilation; HbA1c = glycated hemoglobin; LDL = low-density lipoprotein; NOX = nitrites and nitrates; TNF-α = tumor necrosis factor-α.
proves endothelial function in insulin resistance and diabetes, but they need to be tested in clinical trials with cardiovascular endpoints. Two studies, the Prospective Pioglitazone Clinical Trial in Macrovacular Events and the Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of Glycemia in Diabetes Trial, are currently investigating the effects of pioglitazone and rosiglitazone on cardiovascular events in patients with type 2 diabetes.

### Potential new therapies

Given that L-arginine is a substrate for NOS, it is easy to assume that L-arginine supplementation would activate NOS and produce more NO with greater vasodilation. This hypothesis has been tested in many diverse systems including reversal of endothelial dysfunction associated with diabetes. The improvement in coronary endothelial function by L-arginine supplementation has been associated with a reduction in anginal symptoms, suggesting a reduction in myocardial ischemia in patients with coronary endothelial dysfunction. However, not all studies have demonstrated that L-arginine supplementation has an effect on NO production and vasodilation.

Given that increased oxidative stress plays a pivotal role in the pathogenesis of endothelial dysfunction, administration of antioxidants would be expected to be a reasonable strategy to treat this disorder. Several observations suggest that chronic administration of antioxidants and/or vitamins may be beneficial in improving cardiovascular risk. Preliminary evidence for vitamin E or C and/or other antioxidant therapy to improve vascular function in patients with diabetes has recently emerged. In one study, the effects of short-term dietary supplementation with tomato juice (source of lycopene), vitamin E, and vitamin C on susceptibility of LDL to oxidation and circulating levels of C-reactive protein and cell adhesion molecules were measured in patients with type 2 diabetes. Lycopene administration (tomato juice) and vitamin E were both associated with resistance of LDL to oxidation, but only the latter group showed a decrease in C-reactive protein. Levels of cell adhesion molecules and plasma glucose did not change significantly during the study. In another study, Reaven et al. have demonstrated that vitamin E decreased the susceptibility of LDL to oxidation. This protection occurred in a setting in which glycemic index did not change and protein glycation was not affected. In addition, Pinkney et al. showed that vitamin E supplementation mildly enhances endothelial function estimated by flow-mediated dilation in the forearm in patients with type 1 diabetes.

The hypothesis that also the antioxidant vitamin C could improve endothelial-dependent vasodilation in forearm resistance vessels of patients with non-insulin-dependent diabetes has recently been tested by Ting et al. These investigators demonstrated that in diabetic subjects endothelium-dependent vasodilation was augmented by simultaneous infusion of vitamin C; conversely, in non-diabetic subjects, vitamin C administration did not alter endothelium-dependent vasodilation. The data from this study indeed support the hypothesis that acute administration of vitamin C improves endothelial function associated with the diabetic state, however no insight into chronic effects can be deducted from this study. Nevertheless, no long-term trials of vitamin C supplementation in patients with diabetes have been conducted. Therefore, no conclusion can be drawn at the present time regarding the possible use of this vitamin to prevent endothelial dysfunction and atherosclerosis in patients with diabetes. In addition, although various interventions including antioxidant supplementation were shown to be associated with improvement in endothelial function and epidemiologic data suggest a strong correlation between consumption of dietary antioxidants and cardiovascular disease, randomized trials of antioxidants have yielded disappointing results. Early small studies showed a reduction in cardiovascular events, a finding not reproduced in recent large-scale trials. The reason for the lack of benefits of long-term antioxidant supplementation in the setting of primary and secondary prevention, however, is not clear. Given the strong relation between oxidative stress and endothelial function and the observation that the level of oxidative stress may be an important determinant of clinical events, it may be speculated that the potential of antioxidant supplementation to decrease cardiovascular events may be limited to a subgroup of individuals with elevated levels of endogenous oxidative stress and the very early stages of atherosclerotic disease. Furthermore, recent evidence suggests that certain antioxidants, including vitamin E, may be inappropriate to reduce oxidative stress in vivo or may even be pro-oxidant under certain conditions.

Thus, it is conceivable that combining different antioxidant compounds is essential for an adequate reduction of oxidative stress and its sequelae.

As previously reported an important mediator of diabetic vascular disease is activation of PKC in endothelial cells. In addition, glucose increases superoxide production, which activates PKC-β. These effects of hyperglycemia can be reversed by a PKC-β inhibitor, LY333531. In humans, LY333531 improves endothelial dysfunction induced by hyperglycemia in subjects with normal plasma glucose levels and in patients with diabetes suggesting that selective inhibition of PKC may be a promising approach to improve endothelial function in the diabetic context.

### Conclusions

Diabetes mellitus markedly increases the risk of myocardial infarction, stroke, amputation, and death.
The metabolic abnormalities caused by diabetes induce endothelial dysfunction that predisposes this patient population to accelerated atherosclerosis. Blood pressure control, lipid-lowering therapy, ACE-inhibition and antiplatelet drugs improve endothelial function and significantly reduce the risk of cardiovascular events. Although diabetic patients undergo revascularization procedures because of acute coronary syndromes or critical limb ischemia, the outcomes are less favorable than non-diabetic cohorts. Thus, since most of these patients die from complications of atherosclerosis, they should receive intensive preventive interventions in order to reduce their cardiovascular risk.

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