Prevention Conference VI
Diabetes and Cardiovascular Disease
Writing Group II: Pathogenesis of Atherosclerosis in Diabetes

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Diabetes is associated with increased atherosclerosis and other causes of myocardial dysfunction. The pathogenesis of cardiovascular disease (CVD) in diabetes is multifactorial and can be affected by metabolic and other factors.

Under physiological conditions, the endothelial cell (EC) layer acts as a barrier to separate circulating factors and cells from the arterial intima and media. It also serves as an anticoagulant and fibrinolytic surface producing tissue plasminogen activator, which counters the effects of procoagulant factors such as fibrinogen and plasminogen activator inhibitor-1 (PAI-1). Endothelial cells produce nitric oxide (NO), which is a vasodilator and restrains smooth muscle cell (SMC) migration and proliferation.

Circulating factors (hyperglycemia, increased free fatty acids, altered lipoproteins, and derivatives of glycation and oxidation) and hypertension, all of which are common in diabetes, can damage ECs, leading to their dysfunction. Plasma proteins, among them lipoproteins, cross the endothelial barrier, where they can be retained by subendothelial matrix molecules such as collagen and proteoglycans. These and other matrix molecules are produced by ECs and SMCs. Blood components can be modified, eg, by oxidation and glycation. Modified proteins and lipids can alter EC and SMC gene expression, leading to increased production of procoagulants, adhesion molecules, chemotactic factors, and cytokines. The net effect is the adhesion and penetration of circulating monocytes into the arterial intima, where they undergo differentiation and activation to macrophages. Lipids can accumulate intracellularly after uptake of modified lipoproteins (glycation, oxidation, and glycoxidation) by different scavenger receptors on macrophages and SMCs, as well as extracellularly by attaching to matrix molecules. The resulting lesion is termed the fatty streak.

Both ECs and macrophages produce cytokines and growth factors that permit SMCs to migrate from the media to the intima. In the intima, SMCs proliferate in response to several growth factors. These SMCs and the matrix molecules that they secrete form the fibrous cap, a hallmark of the advanced atherosclerotic plaque. Cell death occurs after exposure of cells to various compounds, eg, glucose, free fatty acids (FFAs), glycoxidation products, and modified lipoproteins in the milieu of the arterial intima. The presence of a large lipid core, necrotic tissue, macrophages, and a thin fibrous cap predisposes to plaque rupture. Conversely, a relative absence of lipid and macrophages and the presence of a thick fibrous cap renders a plaque stable. Thrombosis results from an imbalance between coagulation and fibrinolysis. Plaque rupture and hemorrhage can occur as a result of proteases secreted by vascular cells and from hemodynamic stress, leading to disruption of the fibrous cap. Plaque rupture and thrombosis often underlie clinical events such as acute coronary syndromes, including myocardial infarction. Ruptures also can heal, resulting in complex and sometimes stable plaques.

All of these processes can be potentiated by diabetes, as described below. Diabetes can also affect cardiac function independently of coronary artery atherosclerosis. The processes by which cardiac dysfunction might occur include endothelial dysfunction in the microcirculation, reduced cardiac compliance, microangiopathy, disturbed sympathetic function, impaired calcium cycling, limited myocardial glycolytic oxidative metabolism, and diastolic dysfunction. The pathophysiology underlying diabetic cardiomyopathy requires further investigation.

Metabolic Factors
Metabolic derangements associated with diabetes mellitus include hyperglycemia and its derivatives, advanced glycation end products (AGEs), increased levels of FFAs, and lipoprotein abnormalities. These alterations have been found in persons with type 1 and type 2 diabetes. Most of these abnormalities are also found in individuals with the insulin...
resistance syndrome (IRS), a disorder characterized by hyperinsulinemia, lipoprotein abnormalities, and premature CVD. The IRS, also called the metabolic syndrome, typically precedes and often also accompanies type 2 diabetes. The IRS is also associated with obesity and cigarette smoking. It is being increasingly recognized in adolescents and young adults, largely because of the increasing incidence of obesity and inactivity in the young. A number of epidemiological studies, but not all, have shown a correlation between hyperglycemia and CVD. A debate still exists as to the importance of hyperglycemia per se in the atherosclerotic complications of diabetes independent of commonly associated atherogenic factors. Studies demonstrate a deleterious effect of high glucose levels on ECs and cell function in vitro and in vivo. Hyperglycemia disrupts function by increasing oxidative stress, diminishing NO, which leads to apoptosis and impaired function, and enhancing AGE formation. Increased glycation of circulating lipoproteins occurs with hyperglycemia. In addition, hyperglycemia can alter lipid metabolism in a way that can lead to activation of protein kinase C, alter insulin signaling, and increase adhesion molecule gene expression on ECs, and stimulate inflammation and SMC migration and proliferation. Elevated plasma FFAs levels also may be directly detrimental. The effects of FFAs on vascular cells, however, has not been studied adequately. ECs can utilize FFA as a fuel, and high levels of FFAs can lead to increased oxidative stress and diminished NO synthesis in such cells. High FFA levels can impair fibrinolysis by augmenting concentrations of PAI-1 in blood. Because high levels of FFAs are often found in both types 1 and 2 diabetes and in the IRS, their role in atherogenesis is currently under active investigation.

Lipoprotein abnormalities are more common in people with type 2 than type 1 diabetes. The abnormalities frequently observed in type 2 diabetes include elevation of triglyceride-rich lipoproteins (particularly smaller very-low-density lipoproteins) and low high-density lipoproteins (HDL). Low-density lipoprotein (LDL) levels are similar to those in people without diabetes, but the particles are smaller, denser, and more atherogenic. In association with fasting hypertriglyceridemia, there is an elevation of postprandial triglyceride-rich lipoproteins, a factor emerging as an additional atherogenic determinant. Lipoproteins interact with components of the arterial wall as described above. In addition, HDL can facilitate cholesterol efflux from cells in the arterial wall. Compositional abnormalities of HDL seen in diabetes and IRS, ie, triglyceride enrichment and glycosylation of apolipoprotein A-1, might adversely affect this process.

In addition to effects on atherosclerosis, these metabolic phenomena can alter cardiac function. This may result in part from the hypertension associated with diabetes and IRS. However, other factors, such as directly altered myocardial metabolism of FFAs, glucose, AGEs, and associated gene expression, may contribute.

Oxidation/Glycoxidation

Diabetes can influence the generation of oxygen-centered free radicals by glucose-dependent and -independent mechanisms. Auto-oxidation of glucose is known to generate oxygen-centered free radicals. More recently, it has been shown that cellular oxidation of glucose leads to generation of excess reactive oxygen species in mitochondria. Additionally, increased production of extracellular superoxide occurs in monocytes isolated from persons with diabetes. These free radicals can lead to increased lipid peroxidation, including oxidation of lipoproteins.

Mounting evidence emphasizes that most molecules in the arterial wall can be modified by the spontaneous process of glycation, which is driven by hyperglycemia and by the abundance of other reducing sugars and is typically associated with oxidation in a process termed glycoxidation. This process generates AGEs, which form on most essential elements (namely, proteins, lipids, and nucleic acids); indeed, AGE formation is a process that targets every tissue and cellular compartment, whether in normally aging organisms or more rapidly in the presence of diabetes.

Reactive glycation precursors, whether endogenous or exogenous (eg, dietary components and tobacco), attack proteins and lipids to form complex and irreversible substances that are highly deleterious to the integrity and function of the vessel walls. This can occur in several ways: the first is purely mechanical dysfunction caused by AGE cross-bridges established between macromolecules. This type of fastening of molecules normally made to slide onto each other so as to permit vascular contraction and dilation transforms vessel walls into stiff, inelastic tubes and disturbs basement membrane EC adhesion properties. High blood pressure and other consequences, eg, leakiness, may ensue.

A second form of injury can be caused by AGE accumulation into the vessel wall by way of trapping blood components, eg, immunoglobulins, lipoproteins, and cells, including platelets and NO derivatives. A third and possibly more important form of AGE damage is by a broad range of perturbations of cell function that appears to involve receptor and nonreceptor pathways. Important consequences include cellular activation with the subsequent generation of cytokine/growth factor/matrix and overt growth of vessel wall components. Although AGES arise spontaneously during a lifetime, excess glycemia accelerates this process in diabetes. The vasculature is prone to be “seeded” by these pathogenic substances that trigger local inflammation and hypertrophy. Although the inflammation may be chronic, it can be exacerbated by other inflammatory (oxidized lipoproteins) or infectious (viral, bacterial) pathogens. In fact, because AGE receptors are present constitutively on resting T lymphocytes (CD4, CD8), their activation in the vessel wall may trigger a low-grade, sustained inflammatory state. It should be emphasized that in the presence of sugars or phospholipids, glycoxidation reactions are vastly accelerated. This is consistent with the accelerated rate of atherogenesis in the presence of diabetes and dyslipidemia.

Several but not all studies have demonstrated an increased susceptibility to oxidative modification of LDL in diabetes, in part related to compositional changes, eg, the presence of small, dense LDL. Increased generation of products of lipid peroxidation indicates an excess oxidative burden in diabetes. This may be secondary to reduced antioxidant defens-
es. Oxidative stress can influence the expression of multiple genes in vascular cells, which may accelerate atherosclerosis. These include signaling molecules such as protein kinase C, nuclear factor-kB, and extracellular signal-regulated kinase. Evidence of oxidative damage has been demonstrated in arterial samples obtained from animal models of experimental diabetes and from human subjects with diabetes.

**Endothelial Dysfunction in Diabetes and Insulin Resistance**

Endothelial dysfunction occurs in a wide variety of pathophysiological settings such as diabetes. It is associated with and may precede atherosclerosis. It is accompanied by altered expression of adhesion molecules that affect thrombosis, as described below, and increased permeability (leakiness), which is one of the earliest manifestations of diabetic vasculopathy.

Over the past 10 years, compelling data have demonstrated that endothelial dysfunction, manifested as impaired responses to endothelium-dependent vasodilators (eg, carbachol and methacholine), occurs in both type 1 and type 2 diabetes, as well as in subjects with insulin resistance without diabetes (eg, obese subjects). Endothelial dysfunction is also noted in persons with hypertension and dyslipidemia. Insulin itself exhibits a dose- and time-dependent induction of vasodilatation in healthy humans. This action is impaired in both type 1 and type 2 diabetes and in insulin-resistant subjects. This action of insulin appears to be mediated by its ability to produce NO within the endothelium, ie, it can be blocked by NG-nitro-L-arginine and other competitive inhibitors of NO synthase. An important action of AGEs and oxidation products on the endothelium is their capacity to quench EC-derived NO. This mechanism is thought to be an important contributor to the vasodilatory impairment seen in diabetes, as well as in normal aging, because anti-AGE agents and antioxidants can inhibit it to various yet significant degrees. Insulin has both immediate (seconds to minutes) and long-term (hours) actions on the vasculature. These actions of insulin, or of endothelium-dependent vasodilators, in modulating EC function have been examined directly in the microvasculature of the retina with scanning laser ophthalmoscopy and in the skin by laser Doppler methods.

The fact that endothelial dysfunction can be demonstrated in patients with insulin resistance who do not have diabetes indicates that the abnormality of insulin action is present in a diverse population beyond that of subjects with type 1 and type 2 diabetes. Because most patients with type 1 diabetes are not insulin resistant, hyperglycemia and other aspects of the diabetic milieu per se are implicated in causing endothelial dysfunction. There is evidence that there may be a discrete genetic determinant of this endothelial dysfunction, as judged from studies of first-degree relatives of patients with type 2 diabetes who manifest impaired endothelium-dependent vasodilation in response to methacholine or insulin.

**Inflammation**

In recent years, it has been firmly established that inflammation not only contributes to acute cardiovascular events but is also a key player in the initiation and progression of atherosclerosis. However, little is known about the potentially unique features of this inflammatory process in diabetes.

Several inflammatory markers have been identified in atherosclerotic lesions. Among them are cytokines and growth factors, which are released by activated macrophages that, together with T cells, are major cellular components in atherosclerotic lesions. Cytokines increase the synthesis of platelet-activating factor, stimulate lipolysis, markedly stimulate the expression of adhesion molecules, and upregulate the synthesis and cell surface expression of procoagulant activity in ECs. Thus, cytokines may play crucial roles not only in the initiation but also in the progression of atherosclerotic lesions.

The release of cytokines may be greater in diabetes because some of the processes known to activate macrophages (and therefore the release of cytokines) are enhanced in diabetes. These processes include oxidation and glycoxidation of proteins and lipids. AGE-mediated cytokine release is associated with overproduction of multiple growth factors, including platelet-dependent growth factor, insulin-like growth factor-1, granulocyte/monocyte colony stimulating factor, and transforming growth factor-β, that have broad cellular effects. Similarly, dietary AGEs are capable of triggering blood mononuclear cytokine release, an effect with systemic vascular consequences.

An additional pathogenic phenomenon is increased formation of immune complexes that contain modified lipoproteins. In recent years, immune complexes with modified lipoproteins have emerged as an important risk factor in diabetes.

The fact that immune complexes containing modified LDL presage the development of microvascular complications in type 1 diabetes and are associated with the presence of coronary heart disease in type 2 diabetes. These immune complexes not only induce the release of large amounts of cytokines but also stimulate the expression and release of matrix metalloproteinase-1 without stimulating synthesis of its tissue inhibitor. Whether the immune complexes lead to the production of C-reactive protein, an acute phase reactant protein that has been shown to be an important risk factor for atherosclerosis, is not known. However, this is quite likely, because activation of macrophages by immune complexes leads to the release of tumor necrosis factor, which has been shown to upregulate the synthesis of C-reactive protein.

High levels of soluble immune complexes in patients with insulin resistance. Thus, the increase in immune complexes in diabetes not only may lead to the initiation and progression of atherosclerosis but also may contribute to plaque rupture and cardiovascular events.

Not much is known about plaque differences in subjects with versus those without diabetes. In atherecomy specimens, the cell-rich and necrotic areas are increased in de novo lesions in persons with diabetes. In restenotic areas, an increased content of collagen has been found in vessels from persons with diabetes. 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. 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.\textsuperscript{52} Presently, it remains unclear whether the content of atherosclerotic plaques differs in patients with type 1 versus type 2 diabetes.

Recently, evidence has been obtained to support an increased content of macrophages in the atherosclerotic lesions of persons with diabetes. This is likely a consequence of an increase in recruitment of macrophages into the vessel wall by the higher levels of cytokines present in diabetes. Whether T cells are also increased and show markers of activation in diabetes is not known. Because oxidized LDL peptides are able to activate T cells, and oxidized LDL is increased in diabetes, this is a plausible mechanism.\textsuperscript{53} T cells have been reported to respond to AGEs by releasing interferon-\gamma.\textsuperscript{54} This could be of relevance to the loss of vessel cell homeostasis, particularly as circulating blood cells become activated in diabetes. T-cell activation can inhibit SMC proliferation and collagen biosynthesis, thereby leading to the formation of vulnerable plaques and acute cardiovascular events. Again, perhaps at the center of this controversy is the lack of distinction of plaque pathology between diabetes of types 1 and 2, with the lesion morefibrous and calcified in type 1 diabetes and more cellular and lipid containing in type 2 diabetes.

**Thrombosis/Fibrinolysis**

Diabetes is associated with a prothrombotic state that reflects changes in both thrombosis and fibrinolysis. In part, these abnormalities may result from the insulin resistance that accompanies diabetes, particularly type 2 diabetes. However, they are also observed in type 1 diabetes. Increases in fibrinogen, factor VII activity, and PAI-1 in both plasma and the atherosclerotic plaque; decreases in urokinase in the plaque; and increases in platelet aggregation have all been described in diabetes.\textsuperscript{55} Although the mechanism for the increase in fibrinogen is not known, increases in factor VII activity are related to increases in postprandial hyperlipidemia. PAI-1 overexpression may be attributable to direct effects of insulin and proinsulin.\textsuperscript{56,57} The in vivo effect of very-low-density lipoproteins on PAI-1 synthesis seen in ECs has yet to be documented. The recent report of decreases in PAI-1 and in carotid wall intimal-medial thickness after treatment of patients with type 2 diabetes with thiazolidinediones supports the contribution of insulin resistance to this pathophysiological state.\textsuperscript{58} Whether glucose per se or the accompanying metabolic disturbances are responsible for the requirement of higher doses of aspirin to reduce platelet aggregation in persons with diabetes is not yet clear.\textsuperscript{59}

The prothrombotic state in diabetes appears to contribute to both the pathogenesis of progressive atherosclerosis and acute events in diabetes. Increases in PAI-1 in both plasma and plaque not only decrease vascular SMC migration but are also associated with decreased expression of urokinase within the vessel wall and plaque.\textsuperscript{60} In the setting of a thin fibrous cap, proteolysis in the shoulder regions of plaques, which reflects T-cell and macrophage activation, may precipitate plaque rupture and induce acute syndromes, including unstable angina and myocardial infarction.\textsuperscript{61} The mechanisms underlying the hypercoagulable state in diabetes/insulin resistance require further elucidation, as does the relative importance of the prothrombotic state to the natural history of the atherosclerotic complications of diabetes.

**Major Points and Writing Group II Recommendations**

**Metabolic Stress**

Atherosclerosis and myocardial dysfunction are increased in persons with insulin resistance and/or types 1 and 2 diabetes. A variety of studies suggest that hyperglycemia, increased FFAs, AGEs, and lipoprotein abnormalities contribute to these abnormalities. However, the exact mechanisms and potential benefits of correcting these are unknown. Therefore, we offer the following recommendations:

- Fundamental studies should be conducted of how FFA, hyperglycemia, AGE, and lipoprotein abnormalities lead to atherosclerosis and cardiac dysfunction.
- Clinical trials of pharmacological and nonpharmacological treatment of insulin resistance (with and without diabetes) are needed.
- Studies on how glucose and lipids interact to promote vasculotoxic effects should be performed.
- Studies should be expanded to define and characterize additional environmental factors relevant to CVD in diabetes.

**Oxidative Stress/Glycoxidation**

Increased oxidative stress and glycoxidation play a role in cardiovascular complications of diabetes. Recommendations therefore are as follows:

- Studies should be conducted to determine molecular mechanisms by which oxidative and glyoxidative stress lead to CVD.
- Studies on the relative contributions of exogenous versus endogenous AGE reactants to the vasculopathy of diabetes should be performed.
- Strategies should be developed to inhibit oxidation/glycoxidation, and the effect of these strategies on CVD should be examined.

**Endothelial Dysfunction**

Endothelial dysfunction occurs in IRS and diabetes and may be an important early event in the pathogenesis of CVD. Recommendations therefore are as follows:

- Further studies should be conducted to define the signaling pathway by which insulin, glucose, FFAs, and AGEs affect the NO pathway and endothelial functions.
- Imaging and other methods should be developed that allow study of endothelial structure and function in vivo in humans and rodent models to facilitate functional and genetic studies.

**Inflammation**

Inflammatory processes are important in the pathogenesis of CVD. The importance of inflammatory processes specifically
in diabetes is unclear. Recommendations therefore are as follows:

- Further elucidation of whether there is a unique role for inflammation in CVD in diabetes and states of insulin resistance is required, and if so, the underlying cellular and molecular mechanisms should be determined.
- There should be additional pursuit of plaque differences in patients with type 1 and type 2 diabetes. The relationship between plaque content, risk factors, and the natural history of atherosclerosis between the 2 types of diabetes also requires more insight.

**Thrombosis/Fibrinolysis**

Procoagulant states are observed in people with diabetes and/or insulin resistance. Mechanisms responsible for this and their relative contribution to CVD are uncertain at present. Recommendations therefore are as follows:

- The mechanisms by which diabetes and insulin resistance lead to the procoagulant state should be elucidated.
- Studies, including clinical trials, should be conducted to examine the importance of the procoagulant states to CVD in diabetes.

Each of these areas is relevant to basic science and clinical science.

**References**

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Circulation. 2002;105:e138-e143
doi: 10.1161/01.CIR.000013954.65303.C5

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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