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The Pathologic Continuum of Diabetic Vascular Disease

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Hyperglycemia can promote vascular complications by multiple mechanisms, with formation of advanced glycation end products and increased oxidative stress proposed to contribute to both macrovascular and microvascular complications. Many of the earliest pathologic responses to hyperglycemia are manifest in the vascular cells that directly encounter elevated blood glucose levels. In the macrovasculature, these include endothelial cells and vascular smooth muscle cells. In the microvasculature, these include endothelial cells, pericytes (in retinopathy), and podocytes (in renal disease). Additionally, neovascularization arising from the vasa vasorum may promote atherosclerotic plaque progression and contribute to plaque rupture, thereby interconnecting macroangiopathy and microangiopathy. (J Am Coll Cardiol 2009;53:S35–42) © 2009 by the American College of Cardiology Foundation

Type 2 diabetes mellitus (T2DM) is diagnosed, and hence largely defined, by hyperglycemia. Although this definition has framed the perspective on T2DM, the pathologic imprint of this disease often involves the vasculature, with the hyperglycemia promoting both microvascular and macrovascular complications. Not surprisingly, given complications such as stroke and acute coronary syndromes, much attention has focused on diabetic macrovascular disease. However, the morbidity associated with diabetic microvascular disease, including retinopathy, neuropathy, nephropathy, and limb ischemia, is staggering. Given the impact of diabetic vascular disease, prodigious effort has been directed toward improving vascular outcomes in T2DM. Improving macrovascular outcomes through glucose-lowering interventions has remained a difficult, complicated, and to date, largely unsuccessful enterprise. In contrast, tighter glucose control does limit microvascular disease. These seemingly paradoxical trends force re-examination of the diabetic vascular disease spectrum.

Diabetic Macrovascular Complications

Hyperglycemia can promote vascular complications by multiple postulated mechanisms (Table 1). Increased glucose concentrations can activate nuclear factor-κB (1), a key mediator that regulates multiple pro-inflammatory and pro-atherosclerotic target genes in endothelial cells (ECs), vascular smooth muscle cells (VSMCs), and macrophages. Elevated glucose can foster glycation of proteins, promoting formation of advanced glycation end products (AGEs) protein cross-linking, and reactive oxygen species formation. Hyperglycemia itself can stimulate oxidative stress, which has been strongly implicated as a driving force in atherosclerosis.

Not surprisingly, many early pathologic responses to glucose are manifest in the vascular cells that directly encounter hyperglycemia. The loss of the nonadhesive property of the endothelium, with monocyte adhesion to ECs, is an early atherogenic step. Hyperglycemia increases monocyte adhesion to cultured ECs (2). Hyperglycemia and AGEs can also stimulate EC production of superoxide (1,3), suggesting links between hyperglycemia, AGEs, and oxidative stress. Glucose may also activate matrix-degrading metalloproteinases, enzymes implicated in plaque rupture and arterial remodeling, inducing similar responses in VSMC. Glucose may also stimulate VSMC proliferation, migration, and altered reactivity, for example, through renin-angiotensin activation.

Inflammation has been strongly implicated in both atherosclerosis and T2DM (4–6). Despite this, no single mechanism yet explains why this pattern is found in diabetic patients. Monocytes grown in the presence of high glucose concentrations or isolated from persons with poorly controlled diabetes appear activated (7), with induction of many inflammatory mediators such as protein kinase C and nuclear factor-κB. These targets, as well as others, may promote oxidative stress (8). In vitro studies suggest similar pro-atherogenic effects of hyperglycemia on T lymphocytes, inflammatory cells also involved in atherosclerosis.

Hyperglycemia Versus Dyslipidemia in the Pathogenesis of Atherosclerosis

Attempts to improve cardiovascular (CV) outcomes through glucose control contrast strikingly with the benefits seen in

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Abbreviations and Acronyms

AGE = advanced glycation end product
APC = activated protein C
CV = cardiovascular
EC = endothelial cell
ER = endoplasmic reticulum
PEDF = pigment epithelium-derived factor
PPAR = peroxisome proliferator-activated receptor
T2DM = type 2 diabetes mellitus
VEGF = vascular endothelial growth factor
VSMC = vascular smooth muscle cell

most trials with statins in patients with diabetes. Such data challenge the focus on glucose as the prime determinant of pathologic, or at least vascular, outcomes among patients with diabetes. The relative effects of hyperglycemia versus dyslipidemia in atherogenesis have been difficult to separate. For example, dyslipidemia can be exacerbated by hyperglycemia. At the same time, some data suggest possible independent effects of hyperglycemia on atherosclerosis (9,10). Atherosclerosis was found to develop more rapidly in fat-fed diabetic pigs than in similar dyslipidemic fat-fed pigs without diabetes (9). In low-density lipoprotein receptor-deficient mice with a novel form of diabetes induced by a T-cell–directed viral antigen, consumption of a cholesterol-free diet resulted in hyperglycemia without changes in lipids or lipoproteins (10). Adding increasing amounts of dietary cholesterol led to dyslipidemia, which was the major factor in atherosclerosis progression independent of hyperglycemia in this model (10).

Endoplasmic reticulum (ER) stress may promote atherosclerosis among those with diabetes. All secretory and membrane proteins, many pathogens, and diverse nutrients, including glucose, pass through the ER. Hyperglycemia alone can induce ER stress in multiple tissues, including the liver and fat, activating pathways involved in oxidation and inflammation (11). Thus, ER stress, which can also be stimulated by hypoxia, elevated free fatty acids, and other nonglucose pathways, may promote both diabetes and atherosclerosis (12).

It is of interest to overlay these various postulated mechanisms that promote inflammation and macrovascular disease onto microvascular disease. As noted, in contrast to macrovascular disease, the impact of better glycemic control on microvascular disease is unequivocal. This divergence in clinical experience raises fundamental questions about the nature of microvascular disease, how hyperglycemia modifies the microvasculature, and the implications of differing glucose effects on arterial disease, based on vessel size.

Diabetic Microvascular Complications

Pathological changes in the diabetic microvasculature can alter organ perfusion, particularly affecting organs heavily dependent on their microvasculature supply, namely the retina, kidneys, and peripheral nervous system. The clinical problems associated with these changes—retinopathy, nephropathy, and neuropathy—drive a large burden of T2DM morbidity. Microvascular disease also contributes to peripheral vascular disease, reduced myocardium vascularization, and poor wound healing (13). To some extent, diabetic microvascular disease has been overlooked in terms of its clinical impact and research attention. Further consideration of microvascular disease should begin with an overview of the anatomic nature of the microvasculature.

Structural and Functional Differences: Micro Versus Macro

Microvessels—the smallest functional unit of the CV system—consist of arterioles, capillaries, and venules. These vessels differ significantly from macrovessels with respect to architecture and cellular components. In contrast to larger vessels providing blood to organs, microvessels have specific roles regulating blood pressure and offering nutrient delivery. The microcirculation also has regulatory systems such as vasomotion, permeability, and myogenic responses that can adapt flow to local metabolic needs (14,15). Disturbances in microvascular function may arise before overt hyperglycemia

Table 1 Examples of Mechanisms Implicated in Diabetic Macrovascular Disease

Cellular Players	Mechanisms
Endothelium	NF-κB activation
	Decreased NO production
	Increased reactive oxygen species
	Increased harmful metabolites (peroxynitrite, nitrotyrosine)
	Increased lipid peroxidation products
	Impaired endothelial-dependent relaxation
Monocyte-derived macrophages	Increased IL1β, IL6, CD36, MCP-1
	Induction of protein kinase C
Vascular smooth muscle cells	Increased proliferation
	Increased migration into intima
	Altered matrix components (chondroitin, dermatan sulphate proteoglycans)
	Increased matrix degradation (elastin)
	Increased nonenzymatic collagen glycation

AGE = advanced glycation end products; IL = interleukin; MCP = monocyte chemoattractant protein; NF-κB = nuclear factor-kappa B; NO = nitric oxide.

and vascular pathologic changes (14,15). This timeline underscores the importance of understanding the distinct role of the microvasculature in the natural history of T2DM.

The most consistent structural diabetic microvascular modification is a thickening of the capillary basement membrane, including arterioles in the glomeruli, retina, myocardium, skin, and muscle, resulting in the classic diabetic microangiopathy. This thickening alters vessel function, directly promoting clinical problems like hypertension, reduced wound healing, and tissue hypoxia. Ultimately, in later stages, a frank loss of microvessels occurs, with microvessel drop-out and pruning classically associated with T2DM. The possibility that microvascular pathology contributes to systemic diabetic complications, including macrovascular atherosclerosis, remains an intriguing hypothesis worthy of further exploration.

Mechanisms of Diabetic Microvascular Disease

Glucose and the microvasculature. A linear relationship exists between hyperglycemia and microvascular complications. The impact of improved glucose control in preventing or limiting progression of microvascular disease strongly implicates hyperglycemia in these complications (16). Indeed, the current fasting plasma glucose parameters used to diagnose diabetes derive largely from diabetes-specific microvascular complication data, especially retinopathy (17). More recently, cross-sectional data from the Blue Mountains Eye Study, the Australian Diabetes, Obesity and Lifestyle Study, and the Multi-Ethnic Study of Atherosclerosis showed no uniform glycemic threshold for retinopathy across different populations (18). These data suggest that microvascular complications do not occur at an arbitrary glycemic threshold, a notion also raised for macrovascular disease (18). Ultimately, the interaction between glucose levels and microvascular disease requires a molecular explanation. Interestingly, hyperglycemia-induced molecular responses are especially evident in insulin-insensitive cells that are thus unable to regulate glucose handling. Capillary ECs in the retina, mesangial cells in the renal glomerulus, and neurons and Schwann cells in peripheral nerves can all be categorized in this way (19–21).

Various mechanisms have been proposed for diabetic microvascular complications (Table 2). Of note, as with the macrovasculature, the endothelium is often implicated in diabetic microvascular disease pathways. Together, these data place ECs in the pathologic center of T2DM. Endothelial cells display remarkable heterogeneity in structure and function. The endothelium arising from vessels of different sizes and from different anatomical compartments can express different phenotypic properties in normal and diseased states (22,23). Recently, DNA microarrays examined differences among gene expression profiles of ECs from large and smaller vessels (24). The differentially expressed genes uncovered in this study have diverse, well-established roles in endothelial biology, including extracellular matrix formation, neuronal signaling and migration, angiogenesis, and lipid metabolism (24).

Other specific cell types may also play defined roles in microvascular disease in certain tissue beds. An early, specific retinal change induced by hyperglycemia is the death of microvascular contractile cells known as pericytes. Pericytes provide vessel stability and regulate control of endothelial proliferation and angiogenesis (25). In the kidney, podocytes and ECs form the glomerular capillary, which, together with the basement membrane, constitutes the glomerular filtration barrier. Podocyte injury and loss, which also involves apoptosis, are cardinal features of diabetic nephropathy (26). Many of the mechanisms implicated in microvascular injury are common to ECs, pericytes, and podocytes. Recent clinical evidence reveals that cerebral microangiopathy may play a role in promoting vascular dementia, ventricular hypertrophy, lacunar infarcts, hemorrhage, and may be a predisposing factor for Alzheimer's disease in patients with diabetes (27). It is also worth noting that the heart itself contains a large and extensive microvasculature. Indeed, coronary microangiopathy has been raised as a major complication in diabetics. Compared with controls, hyperemic myocardial flow was decreased 28% in patients with T2DM and macrovascular coronary disease, but was even further reduced (57%) among patients with T2DM who also had evidence of coronary microangiopathy (28,29). As suggested in other tissue beds, glycemic control may influence the degree of coronary microangiopathy (28).

AGEs, strongly implicated in diabetic vascular injury, have been localized to retinal blood vessels in patients with T2DM and correlated with the degree of retinopathy (30). Retinal vascular ECs exposed to AGEs show abnormal endothelial nitric oxide synthase expression (31) and induc-

Table 2 Examples of Mechanisms Implicated in Diabetic Microvascular Disease Secondary to Hyperglycemia

Increased Aldose Reductase Pathway	Protein Kinase Activation	Increased Oxidative Stress	Protein Glycation	Increased Hexosamine Pathway
↑ Sorbitol	↑ VEGF	↑ ROS	↑ AGE	↑ PAI-1
Osmotic cellular damage	↑ ROS		Apoptotic death	Inhibition of eNOS activity
↓ (Na^+ and K^+) ATPase activity	NF-κB activation		NF-κB activation	
↑ NADH/NAD^+	Inhibition of eNOS activity		↑ ROS	
↓ NADPH	↑ Endothelin-1			

ATPase = adenosine triphosphatase; eNOS = endothelial nitric oxide synthase; NAD = nicotinamide adenine dinucleotide; NADH = nicotinamide adenine dinucleotide reduced; NADPH = nicotinamide adenine dinucleotide phosphate reduced; PAI = plasminogen activator inhibitor; ROS = reactive oxygen species; VEGF = vascular endothelial growth factor; other abbreviations as in Table 1.

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tion of vascular endothelial growth factor (VEGF) expression (32). AGEs reportedly signal via the receptor for AGE. In neuronal-associated vessels, the AGE receptor has been localized with its putative ligand N-epsilon-carboxymethyl lysine and nuclear factor-κB, and interleukin-6 (33). The blockade of AGE formation by aminoguanidine improved neural signal transmission in diabetic rats, suggesting this as a therapeutic strategy for diabetic vascular complications (34).

Oxidative stress has been implicated in both microvascular and macrovascular disease. Hyperglycemia promotes formation of reactive oxygen species, which can interact with both deoxyribonucleic acid (DNA) and proteins, causing damage. Mitochondrial DNA may be an especially relevant target (35). Interestingly, reactive oxygen species-mediated cellular damage may be a form of pathologic "memory" in the microvasculature that persists even after glucose normalization, as suggested in human retinal vascular ECs (35). The microvasculature may be more sensitive to such changes simply on the basis of mass. Oxidative stress may also link hyperglycemia with other pathways implicated in diabetic vascular complications, including AGE formation, protein kinase C activation, increased polyol flux, and hexosamine formation (36,37). For example, oxidative stress in response to AGE formation may promote diabetic neurovascular dysfunction (38,39).

Thrombospondin-1, a potent antiangiogenic and proatherogenic protein, has received some attention as a potential mediator linking hyperglycemia to both microvascular and macrovascular disease (40). Glucose alters both cell- and tissue-specific thrombospondin-1 expression and its post-transcriptional regulation in ECs, VSMCs, and fibroblasts (41). In contrast, thrombospondin-1 levels are dramatically decreased by high glucose in microvascular ECs and retinal pigment epithelial cells, making this protein an example of differences between macrovascular and microvascular disease.

Recently, thrombomodulin-dependent formation of activated protein C (APC) was identified as a potential mechanism for hyperglycemia-induced changes in mesangial ECs and podocytes (42). The endothelial thrombomodulin-protein C system is impaired in T2DM, as evident in the increased soluble thrombomodulin and decreased APC levels among such patients (43,44). APC may protect glomerular ECs against apoptosis and has potent antithrombotic and other cytoprotective, fibrinolytic, and anti-inflammatory properties. The APC modulates the mitochondrial apoptosis pathway via the protease-activated receptor-1 and the endothelial protein C receptor in glucose-stressed cells. Loss of thrombomodulin-dependent APC formation interrupts cross-talk between the vascular compartment and podocytes, causing glomerular apoptosis and diabetic nephropathy (42).

Microalbuminuria in T2DM reflects a generalized disturbance of microvascular function related to endothelium-dependent mechanisms. Microalbuminuria may be a marker for the risk of retinopathy, nephropathy, and neuropathy. Interestingly, microalbuminuria may also predict CV disease (45).

The vasa vasorum and neovascularization in diabetes. The vasa vasorum is a network of small vessels normally found only in the adventitia and outer medial layer of larger arteries and the aorta (46). Neovascularization arising from the vasa vasorum may promote atherosclerosis and predict plaque rupture (47). Neovascularization in atherosclerotic arteries occurs by growth from both the adventitial layer outward and the arterial lumen inward, toward the intima (46). The vasa vasorum also provides the arterial wall with a vast absorptive endothelial surface that influences lipid metabolism and delivery, and removal of neurohumoral factors (46).

In T2DM, angiogenesis is increased and associated with plaque rupture (48). Neovasculature microangiopathy may accelerate diabetic atherosclerosis (Fig. 1). The initial angiogenic response in the adventitial vasa vasorum appears stimulated by hypoxia and ischemia, perhaps through increased hypoxia-induced factor-1 and VEGF action (49). VEGF also increases vascular permeability to macromolecules, monocyte chemotaxis, and tissue factor production, possible contributors to microvascular complications (50,51). VEGF is also associated with diabetic nephropathy (52). Increased vascular permeability instigates an inflammatory response, with recruited monocyte-macrophages serving as a source of VEGF (53–56). Conversely, VEGF treatment may limit diabetic neuropathy by restoring microcirculation in the vasa nervorum, as suggested by rodent VEGF gene transfer experiments (57).

In the eye, pigment epithelium-derived factor (PEDF) may offset VEGF action, providing another example of tissue-specific settings in microvascular disease. PEDF is a neurotrophic factor and a potent angiogenic inhibitor (58). In proliferative diabetic retinopathy, VEGF levels are increased while PEDF levels are decreased (59). Decreased PEDF levels may also contribute to diabetic nephropathy. Other growth factors may foster proliferative retinopathy, including insulin-like growth factor 1, basic fibroblast growth factor, and hepatocyte growth factor (60,61).

Additional contributors to diabetic microvascular disease. Dyslipidemia is strongly associated with the development and progression of microvascular disease. Increased levels of dense low-density lipoprotein, as well as low-density lipoprotein modified by glycation and oxidation, may foster retinopathy, neuropathy, and nephropathy; and attenuated levels and function of high-density lipoprotein have also been linked to retinopathy (62–65). T2DM is often characterized by abnormal very-low-density lipoprotein and triglyceride levels. Elevated very-low-density lipoprotein and triglyceride levels appear involved with retinopathy and albuminuria. This may be due to changes in the function of lipoprotein lipase, a key enzyme in triglyceride hydrolysis, which acts in the microvasculature. Interestingly, lipoprotein lipase action can generate natural peroxisome proliferator-activated receptor (PPAR) ligands as we and others have found (66,67). Perhaps lipoprotein

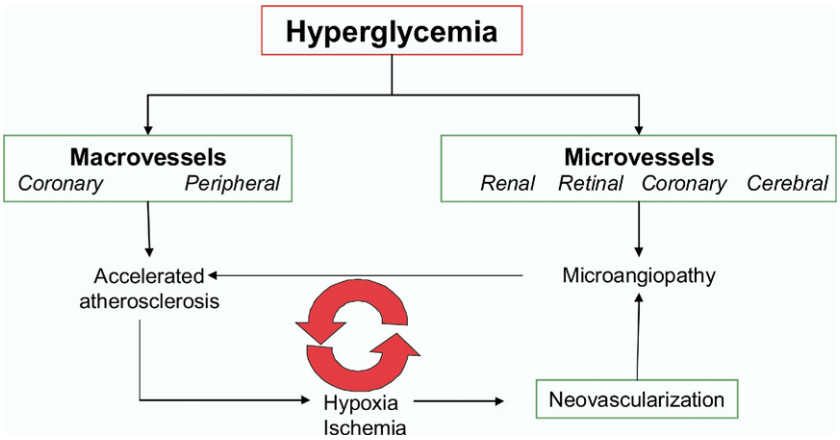

Figure 1 **Intersection of Microangiopathy and Macroangiopathy in Accelerated Atherosclerosis**

In type 2 diabetes mellitus, both angiogenesis and microangiopathy are increased and may contribute to accelerated atherosclerosis and the development of vulnerable plaque. Hyperglycemia is a driving force in both large- and small-vessel disease. Indeed, the 2 disorders may be interconnected, with microvascular disease promoting atherosclerosis through processes such as hypoxia and changes in the vasa vasorum.

lipase dysfunction in T2DM promotes microvascular disease through a loss of endogenous PPAR agonists. Potentially consistent with this, in the FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) study, the PPAR-α agonist fenofibrate reduced the need for laser treatment for diabetic retinopathy as a tertiary end point (68). Of note, this effect did not appear to be mediated by improved lipid, glycemic, or blood pressure profiles, leaving the mechanism involved unclear. PPAR-α agonists inhibit the VEGF pathway, which may promote angiogenesis, inflammation, and cell migration (69,70); they also regulate retinal EC survival, limit apoptotic cell death (71), and improve vascular reactivity (70). In the DAIS (Diabetes Atherosclerosis Intervention Study) trial, the improved lipid profiles with fenofibrate in patients with T2DM were associated with reduced progression to microalbuminuria (72).

In animal studies, thiazolidinediones reportedly decreased proteinuria or delayed progression to nephropathy; results were independent of insulin sensitization, glycemic control, and lipid metabolism, but were associated with reductions in blood pressure (73). In 6 randomized, active-controlled trials up to 12 months in duration with either pioglitazone or rosiglitazone, significant 10% to 30% reductions in albumin-to-creatinine ratio were demonstrated (74,75).

Adiposity may promote microvascular disease. Increased fat mass and resistance to insulin-mediated inhibition of lipolysis increase elevated free fatty acid levels, which can directly impair microvascular function and increase diabetic microangiopathy (76). Increased visceral fat is a source of inflammatory mediators such as tumor necrosis factor-α, interleukins, and the pro-coagulant plasminogen activator inhibitor-1. In addition to increasing C-reactive protein levels and oxidative stress, these mediators also stimulate endothelial degradation and leukocyte adhesion, with the possible obstruction of microvessels (77,78).

Inflammation also promotes diabetic retinopathy, nephropathy, and neuropathy (79,80). Leukocyte adherence and accumulation within the retinal vasculature is an early change in experimental diabetes (81,82). Overexpression of adhesion molecules (83) and certain chemokines (84) may promote diabetic nephropathy. In diabetic retinopathy, this may result from elevated tumor necrosis factor-α levels (85). In addition to promoting leukocyte infiltration and activation, tumor necrosis factor-α may also enhance microvascular cell apoptosis (86). In keeping with these observations, tumor necrosis factor-α inhibition decreases leukostasis (87). Consistent with their opposing roles discussed earlier, VEGF can also trigger inflammation (87), whereas PEDF appears to limit it (88).

Intersection of Diabetic Microvascular and Macrovascular Disease

T2DM may be best characterized by its complexity. Arising over decades, T2DM involves multiple pathologic forces resulting in a range of clinical issues. This complexity is evident in the problems of diabetic macrovascular and microvascular disease. Diabetic subjects with microvascular complications, ~25% to 30% of all those with diabetes, appear particularly prone to accelerated atherosclerosis and premature death. Neovascularization arising from the vasa vasorum may interconnect macroangiopathy and microangiopathy (Fig. 1).

Why the benefits of glycemic control are readily apparent in microvascular but not in macrovascular outcomes remains obscure. The similarity and differences between diabetic

microvascular and macrovascular disease, including differing responses to therapeutic interventions, remain important, unresolved issues in the field of T2DM; clarity in these areas could lead to treatments that improve outcomes in patients with diabetes.

The challenge to better understand how all forms of vascular disease occur in T2DM and how to intervene allows us to refocus on perhaps the most obvious clinical issue at hand: implementing treatments known to improve T2DM outcomes. Microvascular disease is significantly improved by tighter glycemic control, which should be implemented as early as is safely possible and maintained for as long as possible. Such interventions can significantly reduce a large burden of disease. In terms of macrovascular disease, diabetic control involves appropriate control of blood pressure as well as lipids. These steps should also be taken early in the natural history of T2DM. While we wait for greater insight and better therapeutic options in T2DM, these simple steps would have a major impact on improving outcomes for millions of people.

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