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Pathophysiology of Vascular Dysfunction in Diabetes

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Diabetes mellitus (DM) was likely first described about 3500 years ago and given its name about 2200 years ago by Demetrios of Apamaia. The word "diabetes" derives from the Greek "diabeinein" or "siphon," a word that captures its association with excess urination. Although DM has been primarily regarded as a disorder of glucose metabolism and homeostasis, it has more recently been viewed as a constellation of metabolic disturbances, including abnormalities of carbohydrate metabolism, adipose storage, lipid metabolism, and protein biochemistry. Although commonly characterized as a disease of impaired skeletal muscle glucose uptake, DM adversely affects hepatic, muscle, adipose, and vascular function. Indeed, it is this last effect that may represent the greatest mortality hazard in this population. Among the classical cardiovascular risk factors for myocardial infarction (MI), only DM carries the same risk of MI as survivors of MI.[1] DM creates an environment adverse to vascular function through a wide variety of dysmetabolic assaults.

Secular trends in the United States have increased the level of attention paid to DM and its sequelae. The incidence of DM is increasing rapidly as a result of aging and an ever more obese population.[2] Indeed, between 2000 and 2010, the number of patients with DM is expected to increase by 23% in the United States and 46% around the world.[3] The fate of these patients is linked directly to atherosclerosis. Half of all patients with type 2 DM have evidence of coronary artery disease when they present with DM[4] and the vast majority of DM-related hospital admissions are for atherosclerotic vascular disease.[5] DM increases the frequency of stroke, heart attack, and amputation 2- to 4-fold, putting these patients at great risk.[6]

The endothelium

There are many physiological impairments that plausibly link DM with a marked increase in atherosclerotic vascular disease, including platelet hyper-reactivity, a tendency for negative arterial remodeling, impaired fibrinolysis coupled with a tendency for thrombosis and coagulation, increased inflammation, and endothelial dysfunction.[7,8] In contrast to the other factors on this list, endothelial dysfunction may be an important nexus of dysfunction in DM, linking each of these pathological manifestations. Endothelial dysfunction, present at disease onset, may be the forme fruste of atherogenesis that is present throughout the course of DM and associated with late-stage adverse outcomes.

The concept of an active endothelium, integral to blood vessel function, is a relatively new one. In contrast to the supposed inert inner vascular barrier, Furchgott and Zawadzki demonstrated that vascular tone is regulated by the endothelium.[9] Since that seminal observation, investigations have revealed that vascular endothelium, a crucial regulator of vascular homeostasis,

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participates importantly in the control of:

- vascular tone and blood flow
- coagulation and thrombosis
- nutrient delivery and waste removal
- inflammation
- vascular smooth muscle cell growth and migration
- leukocyte attraction and diapedesis.

The development of endothelial dysfunction may portend an environment that allows the development of vascular disease, providing a link between DM and microvascular and macrovascular disease (eg, retinopathy, nephropathy, MI, stroke, and amputation).

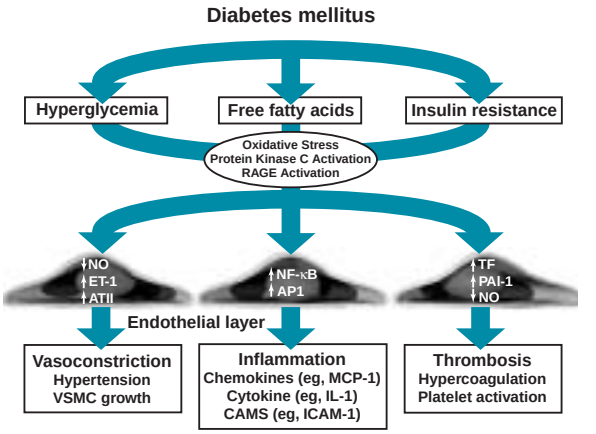
Endothelial vascular regulation occurs through the production and elaboration of autocrine and paracrine factors that regulate both the function and structure of vessel wall constituents. The endothelium maintains a balance between vasodilation and vasoconstriction, coagulation and anticoagulation, and leukocyte attraction and diapedesis. Of the many factors regulated by the endothelium, perhaps the best characterized is nitric oxide (NO).[10] Produced by nitric oxide synthase (NOS) III or endothelial NOS (eNOS), NO is a potent vasodilator, platelet antagonist, and anti-inflammatory agent.[11] Its release is modulated by a wide array of chemical and biophysical stimuli, allowing its fine modulation.

Other important endothelium-elaborated mediators of vascular tone and function include prostanoids, endothelin, and angiotensin II. When patients have a risk factor for vascular disease, such as DM or atherosclerosis *per se*, the bioavailability of NO is attenuated. Thus, stimuli that commonly cause the release of NO are no longer able to produce vigorous vasodilation.

Because of the evanescence of NO, investigating its bioavailability in humans is done indirectly by taking advantage of its vasodilatory properties. Endothelial NOS may be stimulated by the infusion of agonists, such as acetylcholine or one of its congeners – methacholine, bradykinin, serotonin, or substance P – all of which are receptor-mediated modulators of NO release.[12] This method is typically used to examine resistance in arteriolar function.

Peripheral conduit vessel NO bioavailability can be studied by the response to reactive hyperemia.[13] In this experimental model, a sphygmomanometric cuff is placed on the arm, inflated to suprasystolic pressure for 5 minutes, and released. Upon release, as a result of reactive hyperemia, blood flow into the limb beyond the cuff increases 4- to 7-fold. This increase in blood flow and shear stress across the segment of interest causes the release of NO, dilating the artery 60 to 70 seconds after cuff release. Thus, the increase in size (diameter) of the artery at one minute after restoration of flow is an index of NO bioavailability.[14] This examination is typically performed in the brachial artery, but has been reported in other conduit vessels. In humans, endothelial dysfunction is typically characterized by an attenuation of this vasodilatory response or even frank vasoconstriction.

Figure 1: The fundamental metabolic abnormalities in diabetes mellitus activate adverse protein kinase C, increase the production of advanced glycation endproducts, and amplify superoxide anion production. In turn, endothelial homeostatic mechanisms are co-opted, including vasodilation, attenuation of inflammation, and platelet antagonism[8]



ET-1 = endothelin; AT II = angiotensin II; NF-kB = nuclear factor kappa B; AP-1 = activator protein-1; TF = tissue factor; PAI-1 = plasminogen activator inhibitor-1; IL-1 = interleukin-1; ICAM-1 = intracellular adhesion molecule-1; NO = nitric oxide; VSMC = vascular smooth muscle cell; RAGE = receptor to advanced glycation endproduct

Mechanisms of endothelial dysfunction in DM

The mechanisms underlying impaired endothelial function in patients with type 2 DM are varied, but likely include metabolic derangements such as hyperglycemia, excess liberation of free fatty acids (FFAs), and insulin resistance (Figure 1). These derangements increase NO scavenging by increasing the activation of protein kinase C, augmenting production of reactive oxygen species (ROS), and uncoupling eNOS.

Hyperglycemia

The elevation of blood glucose was the first recognized abnormality in DM. The endothelium, a sensitive sensor for elevations in glucose, becomes rapidly dysfunctional in healthy human subjects. Dysfunction in response to hyperglycemia is seen as early as 6 hours.[15,16] The rapidity of these functional changes indicates that the endothelium is an early indicator of hyperglycemia. Furthermore, it may convey these changes so rapidly, in contrast to other cellular components of the vasculature, because of the persistent expression of glucose transporter 1 in endothelial cells despite ambient hyperglycemia.[17] The intracellular glucose concentration of vascular endothelial cells echos that in the extracellular environment. In contrast, vascular smooth muscles cells maintain a normal intracellular glucose concentration by limiting glucose transport.[18]

The increased production of pathogenic reactive oxygen species (ROS), (eg, superoxide anion), represents a central abnormality caused by hyperglycemia. In contrast to other risk factors, DM co-opts the protective affects of endothelial cells and causes them to become the primary source of vascular oxidative stress.[19] In the setting of other risk factors, endothelial cells minimize oxidative stress.

DM creates a cascade, employing an ever increasing number of cellular components in the production of ROS:

• Beginning with the mitochondria, hyperglycemia attenuates the donation of electrons for ATP generation, shifting the electron transport chain towards generation of superoxide anions.[20]

• Mitochondrial production of superoxide anions activates protein kinase C and NAD(P)H oxidase, increasing the production of cytosolic superoxide anions.[21]

• Increased superoxide anions scavenge NO to form peroxynitrite, which oxidizes the eNOS co-factor tetrahydrobiopterin and triggers preferential production of superoxide anion instead of NO by eNOS.[22]

• Extracellular production of superoxide anions also increases as a result of increased xanthine oxidase liberation (likely from the liver).[23-25]

We have demonstrated that hyperglycemia impairs endothelial function in 6 hours[15] and that this impairment can be reversed with infusion of an antioxidant.[16] Moreover, we showed that inhibition of protein kinase C beta prior to hyperglycemia prevents vascular dysfunction,[26] confirming the importance of ROS and protein kinase C in the early development of hyperglycemia-induced endothelial dysfunction in humans.

Hyperglycemia also increases other sources of oxidative stress, including the intracellular production of advanced glycation endproduct (AGE) and activation of the endothelial receptor to AGE (RAGE). Functional abnormalities arise as a response to intracellular protein modification and activation of RAGE.[27] AGEs can produce ROS, *per se*, and increase intracellular enzymatic production of ROS via activation of RAGE.[28,29] Increases in glucose concentration also heighten diacylglycerol concentration,[30] causing the preferential activation of protein kinase C isoforms ß and δ.[31,32] Controlling glycemia eliminates activation of protein kinase C, thus implicating the importance of this pathway of activation *in vivo*.[33,34]

Increased free fatty acid concentration

The increase in FFA liberation from adipocytes also augments the oxidative stress burden and diminishes NO bioavailability.[35] Arising from the increased release from adipose tissue and decreased skeletal muscle uptake,[36] increased plasma FFA heightens oxidative stress by augmenting small, dense, oxidized low-density lipoprotein (LDL),[37,38] and by directly affecting the endothelium.[39] In the endothelium, analogous to hyperglycemia, FFA induces membrane translocation and activation of protein kinase C, increases endothelin-1 production, and increases superoxide anion production.[35,39-42] The adverse effects of FFA on endothelial function have been demonstrated in humans. Infusion of FFA to postprandial levels impairs endothelial dysfunction in a matter of hours and this impairment can be reversed with infusion of an antioxidant.[35,43]

Insulin resistance

Insulin resistance often precedes increases in glucose by years to decades and plays a key role in atherogenesis. Peripheral resistance to insulin precedes beta cell failure that produces overt hyperglycemia. Although exemplified by impaired skeletal muscle glucose uptake, insulin resistance also occurs in liver, adipose tissue, and the endothelium. The extent of insulin resistance correlates both with glucose disposal and endothelium-dependent vasodilation.[44] Endothelial dysfunction occurs in the early stages of DM,[45-47] prior to evidence of microvascular disease.[48] Although there is a clustering of cardiovascular risk factors (the metabolic syndrome) prior to DM onset, it is likely that insulin resistance contributes additively to vascular risk. This pathobiological link between insulin resistance and endothelial dysfunction hints that improvements in insulin resistance may result in improved endothelium-dependent vasodilation. Support for this concept has been demonstrated in humans with the insulin-sensitizing agents, troglitazone and metformin.[49,50]

Endothelial dysfunction in diabetes

The metabolic disturbances described above impair endothelial cell activity and make the vascular environment more favorable for development of atherosclerosis. Diabetes compromises each anti-atherosclerotic process, including vascular tone regulation, regulation of vessel-leukocyte interactions, and platelet antagonism, creating a permissive environment for the progression of atherogenesis.

Vasomotor function

Augmented production of ROS, especially superoxide anions, decrease the bioavailability of endothelium-derived NO. Superoxide anions scavenge NO to form peroxynitrite.[51] Endothelium-dependent, NO-mediated vasodilation is attenuated in resistance and conduit vessels in patients with type 1 and type 2 DM,[45,52-54] and oxidative stress inhibits the production of compensatory vasodilators (eg, prostacylin) to further limit vasorelaxation.[55]

Paralleling the decrease in vasodilator mediators, DM heightens production of vasoconstrictor peptides, including endothelin-1 and angiotensin II. A wide variety of insults

in DM – including insulin resistance and hyperglycemia, increased oxidative stress, protein kinase C membrane translocation and activation,[31] and RAGE ligand-receptor interaction[56] – combine to increase endothelin production.[41,42] Endothelin causes vascular smooth muscle contraction with an increase in vascular tone, stimulates angiotensin II production and vascular smooth muscle proliferation, and increases salt and water retention.[57]

Inflammation

Inflammation seems to fuel the atherogenic process[58,59] and is strongly linked to DM and insulin resistance.[60,61] Atherosclerosis is initiated with T-lymphocyte migration into the vascular intima.[59] These cells produce cytokines and chemokines and recruit monocytes and vascular smooth muscle cells into the nascent plaque. The monocytes and vascular smooth muscle cells scavenge oxidized LDL, become foam cells and, when amassed, become fatty streaks.[58] Endothelial dysfunction enhances each of these early atherogenic processes through activation of inflammatory transcription factors, such as nuclear factor kappa B (NF-kB).[62-64] These factors increase gene expression of proinflammatory cytokines and chemokines, production of leukocyte-adhesion molecules, and inflammatory mediator content within atherosclerotic lesions – processes that foster atherogenesis.[65-67] In the Third National Health and Nutrition Examination Survey (NHANES III), both glycemia and insulin resistance correlated directly with markers of inflammation, demonstrating a link between the dysmetabolism of DM and poor vascular outcomes.[68]

Similarly, improvements in insulin resistance and glycemic control reduce inflammation.[69,70] These benefits are translated to the vasculature. Reductions in inflammation through medication[71] or by reducing visceral adiposity,[72] improve endothelial function and soluble markers of endothelial cellular activation.

DM exacerbates the late stage of atherosclerosis as well. Mature atherosclerotic plaque is characterized by a lipid gruel separated from the vessel lumen by a fibrous cap. DM increases endothelial cell matrix metalloproteinase production, which decreases synthesis of vascular smooth muscle cell collagen.[73,74] As collagen diminishes and fibrous cap collagen metabolizes, the risk of plaque rupture increases.

Thrombosis

The tendency for thrombosis is an important consideration in determining the clinical severity of plaque rupture through modulation of thrombus formation and arterial occlusion. Diabetic endothelial cells produce tissue factor, a powerful coagulant found in atherosclerotic lesions.[75] Moreover, the dysfunctional endothelium has attenuated anticoagulation as well. Thrombomodulin expression (a cell-surface based anticoagulant) is decreased, while production of plasminogen activator inhibitor-1 (a fibrinolytic antagonist) is increased. Moreover, the combination of reduced NO and prostacyclin enhances platelet activation and aggregation.[76-78] The increase in coagulation and thrombosis potentiate thrombus formation after plaque rupture and make the development of arterial occlusion and clinical events more likely.

Conclusion

The last 25 years have clearly demonstrated the central role of the vascular endothelium in maintaining vascular health, as well as the effects of endothelial dysfunction. DM impairs every homeostatic mechanism employed by the endothelium to prevent the development of atherosclerosis. Investigations in humans with DM have made clear the importance of many of the pathogenic processes elucidated by basic science investigation. As our understanding of human vascular function progresses, new therapeutic strategies may be developed to reduce the cardiovascular risk suffered by patients with DM.

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