

The Pathogenesis of Diabetic Atherosclerosis

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Keywords

Diabetes • Atherosclerosis • Hyperlipidemia • Insulin resistance • Reactive oxygen species

Introduction

Patients with diabetes mellitus (DM) have an over tenfold risk for cardiovascular disease in their lifetime [1]. In the United States, 77% of diabetes-related hospital admissions are for cardiovascular complications. A key feature of diabetes contributing to this is the development of an accelerated atherosclerosis [2]. Cardiovascular disease is one of the most morbid complications of DM with men and women being equally at risk, essentially eliminating the protection against cardiovascular disease characteristic of premenopausal women. DM predisposes to higher rates of coronary artery disease (CAD), cerebral vascular disease, and peripheral arterial disease (PAD). Aggressive blood sugar control has been shown to decrease some cardiovascu-

lar sequelae in diabetics, particularly in type I DM; however it does not eliminate all risk and intensive glycemic control for type II diabetics has not proven to be beneficial and may even be detrimental [1, 3].

CAD is the most morbid cardiovascular complication of DM with a two- to fourfold increased risk [4]. Compared to cardiovascular disease in nondiabetics, diabetic patients have a greater overall coronary plaque burden and a higher rate of multivessel disease. The proportion of stenotic segments is directly proportional to the duration of disease [5]. In combination, these factors place diabetic patients at greater risk for myocardial infarction (MI). In fact, diabetics without a prior MI are at equal risk for MI as nondiabetics with a prior MI. After MI, complications and death are higher in DM. The increased risk also extends to those undergoing cardiac procedures. After percutaneous coronary intervention (PCI), diabetic patients are at both higher risk for death and need for reintervention [6]. Diabetic patients who undergo coronary artery bypass grafting (CABG) are at higher risk for both complications and death, particularly in those with insulin-dependent type II DM, with no benefit seen in those who have had tight postoperative glycemic control [7, 8].

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Similarly to CAD, DM also carries a two- to fourfold increased risk of PAD. The distribution of lower extremity lesions in DM shows a higher propensity of atherosclerotic disease in the deep femoral artery, as well as in all vessels below the knee. Not surprisingly, DM is the leading risk factor for on-traumatic lower extremity amputations and diabetic patients have a higher frequency of infrageniculate arterial interventions [4, 9]. In the presence of DM, occlusive lesions are more common than stenoses [10]. Diabetic patients do not have a higher risk of graft failure, death, or cardiovascular complications after lower extremity bypass, nor do they have higher rates of failure after peripheral percutaneous intervention [10–12]. DM also increases the risk of extracranial cerebral vascular disease with a threefold increased risk of stroke and a greater rate of post-stroke complications, including recurrent stroke and death [4]. Diabetics undergoing carotid endarterectomy (CEA) are more likely to be younger and have concurrent CAD compared to nondiabetics. However, DM is not an independent predictor of perioperative MI, stroke, or death [13].

The effects of diabetes on the vasculature are quite extensive as diabetes affects not only the endothelium and smooth muscle cells, but also platelets, lipoproteins, local vasoactive substance production and function, clotting factors, triglycerides, as well as local arterial response to hypoxia and new collateral vessel formation [4]. The pathogenesis of diabetic atherosclerosis involves not only the direct effects of chronic hyperglycemia, but also insulin resistance, nonesterified free fatty acid (NEFA) production, dyslipidemia, hypercoagulability, and impaired response to injury [4, 14]. It is this widespread dysfunction that makes the side effects so deleterious and the treatment so difficult.

Histology

Arteries are composed of three layers—the tunica intima, media, and adventitia. The tunica intima is the innermost layer with the luminal side being composed of a single layer of endothelial cells. The next layer of the intima consists of an extracel-

lular connective tissue matrix composed primarily of proteoglycans and collagen. Surrounding the intima is an internal elastic lamina that is composed of elastic cells of varying thickness depending on the vessel size. The tunica media is the next layer composed of primarily vascular smooth muscles cells and it is the thickest layer of the blood vessel. This layer is surrounded by the external elastic lamina, which separates the tunica media from the tunica adventitia, the outermost layer of the vessel wall. This layer is mainly composed of collagen with interspersed fibroblasts and vascular smooth muscle cells [15].

The development of diabetes-related atherosclerosis follows the same histologic course as atherosclerosis in nondiabetic patients. This includes endothelial injury, smooth muscle cell proliferation, foam cell development and infiltration, platelet activation, and increased inflammation. Sites of lesions are determined by altered hemodynamic forces and external sources of injury to the endothelial cells. Increased endothelial permeability leads to the retention of deleterious low-density lipoproteins (LDL) that interact with the underlying extracellular matrix (ECM). This interaction retains the LDL in the vessel wall where it can undergo oxidation by reactive oxygen species (ROS). This oxidized LDL can then stimulate the overlying endothelial cells to upregulate cellular adhesion molecules, chemotactic proteins, growth factors, and inhibit nitric oxide (NO) production. These activities recruit monocytes and macrophages, which interact with highly oxidized aggregated LDL to form foam cells. Pro-inflammatory cytokine production by activated macrophages stimulates proliferation of vascular smooth muscle cells (Fig. 2.1). Intimal smooth muscle cells subsequently produce an ECM that gives rise to a fibrous cap. The resulting complex plaque is vulnerable to destabilization, rupture, and superimposed thrombosis leading to an acute vascular occlusion (Fig. 2.2) [15].

Atherosclerotic plaques in the presence of diabetes generally have increased calcification, necrotic cores, receptors for advanced glycosylation endproducts (RAGE), and macrophage and T-cell infiltration. There is also a higher incidence of healed plaque ruptures and vascular

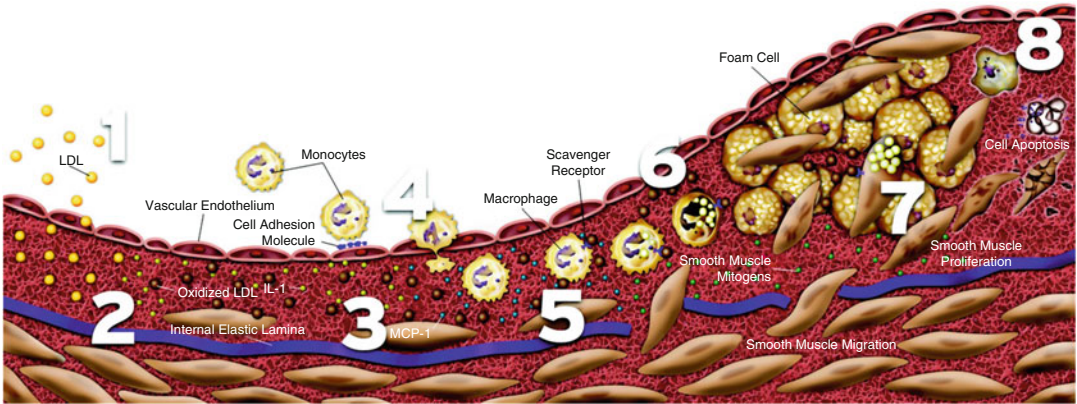


Fig. 2.1 The stages of development of an atherosclerotic plaque. (1) LDL is taken up by the endothelium. (2) Oxidation of LDL by macrophages and VSMCs. (3) Release of growth factors and cytokines. (4) Attraction of additional monocytes. (5) Foam cell accumulation. (6) SMC proliferation.

(7, 8) Formation of plaque [reprinted from Faxon DP, Fuster V, Libby P. Atherosclerotic vascular disease conference: Writing Group III: Pathophysiology. *Circulation*. 2004;109(21):2617–25. With permission from Lippincott Williams & Wilkins]

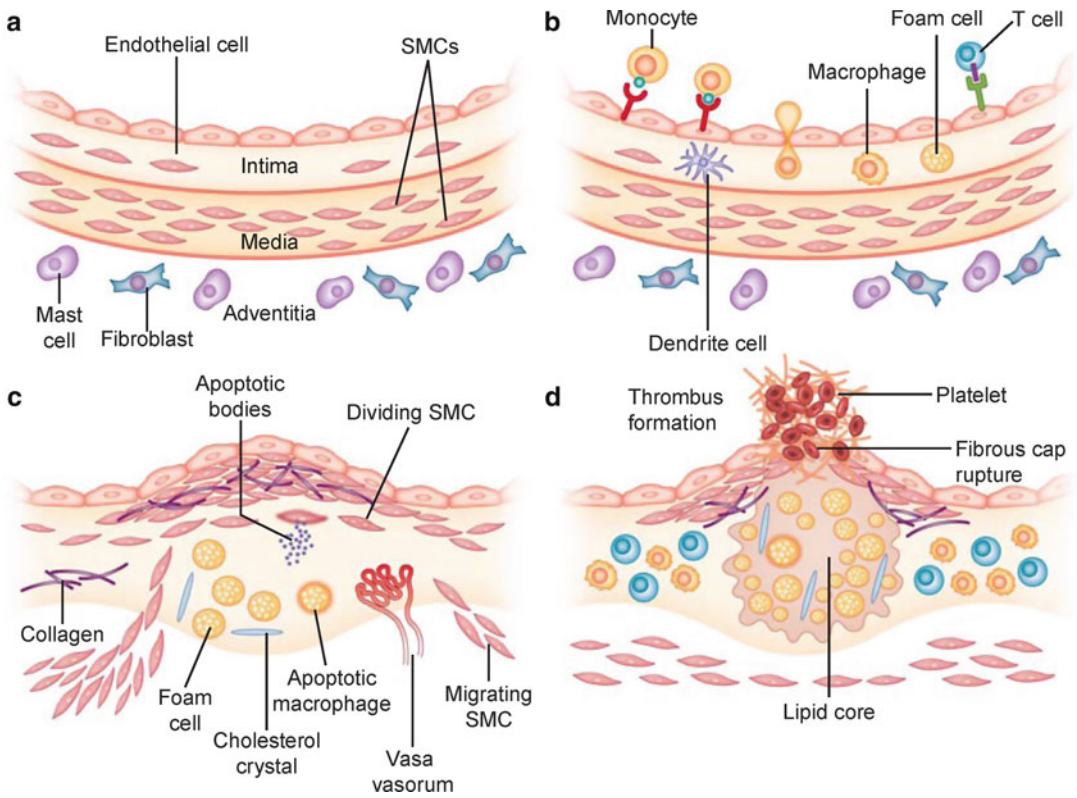


Fig. 2.2 Development of atherosclerotic plaque with superimposed thrombus [reprinted from Libby P, Ridker PM, Hansson GK. Progress and challenges in translating

the biology of atherosclerosis. *Nature*. 2011;473(7347): 317–25. With permission from Nature Publishing Group]

remodeling [16]. These features can potentially contribute to the more severe atherosclerosis and a higher incidence of acute adverse events.

Endothelial Cells

Endothelial cells synthesize multiple regulatory substances including NO, prostaglandins, angiotensin, and endothelin-1 (ET-1), and can upregulate adhesion molecules for interactions with neutrophils and platelets. These mediators help regulate vasodilation and vasoconstriction, hemostasis, and inflammation on the vessel surface and within the wall [2, 4]. Nitric oxide (NO) is anti-atherogenic, produced by the action of endothelial nitric oxide synthase (eNOS), and is a key contributor to vasodilation and prevention of platelet aggregation. Endothelial cell injury and dysfunction is thought to be the sentinel event in the development of atherosclerosis [17]. An intact endothelium normally inhibits platelet activation and inflammation by reducing upregulation of platelets and leukocyte adhesion molecules and subsequent migration through the vessel wall, diminishing vascular smooth muscle cell proliferation and migration [4]. Endothelial cells are particularly susceptible to accumulation of glucose seen in hyperglycemia and to many of the secondary side effects of DM including insulin resistance [18].

Vascular Smooth Muscle Cells

Vascular smooth muscle cells primarily comprise the tunica media and are responsible for contraction and relaxation to vary the caliber and pressure of blood vessels. Vessels in higher pressure systems tend to have more smooth muscle cells than those in lower pressure systems. Contraction and relaxation are primarily regulated by the sympathetic nervous system. Autonomic function is altered in DM resulting in abnormal vasodilation and vasoconstriction in response to local factors. The medial layer of smooth muscle cells proliferates into intimal lesions and atherosclerotic plaques develop with smooth muscle cells as the source of collagen to strengthen the plaque [4].

Monocytes

Monocyte activation and transformation into macrophages are a key step in the atherosclerotic and inflammatory process. One of the earliest events in the pathogenesis of atherosclerosis is lipid accumulation in monocytes through uptake of modified or oxidized low-density lipoprotein (LDL) leading to the foam cell infiltration in the arterial wall [15]. Activation of macrophages with subsequent release of smooth muscle growth regulatory molecules in diabetic lesions contributes to vascular smooth muscle cell proliferation [19].

Hyperglycemia

Hyperglycemia increases the production of reactive oxygen species (ROS) as a consequence of mitochondrial dysfunction, which in turn promotes atherosclerotic lesion formation by upregulation of protein kinase C (PKC), activation of the hexosamine and polyol pathways, and accumulation of advanced glycation endproducts (AGE) with upregulation of RAGE receptors. In many tissues, glucose uptake is mediated by insulin-independent glucose transporters (GLUT). Therefore, a rise in intracellular glucose concentrations parallels serum levels [20].

Mitochondria

Mitochondrial dysfunction is one of the initial pathophysiologic events observed in hyperglycemia. Increased glycolysis during aerobic metabolism in the setting of hyperglycemia generates nicotinamide adenine dinucleotide (NADH) and pyruvate. Pyruvate is then transported into the mitochondria where it enters the tricarboxylic acid (TCA) cycle generating three molecules of carbon dioxide, four molecules of NADH, and one molecule of flavin adenine dinucleotide (FADH_2). In mitochondria, NADH and FADH_2 donate electrons for the generation of adenosine triphosphate (ATP) through oxidative phosphorylation in the electron transport chain (ETC). The ETC progresses sequentially through four

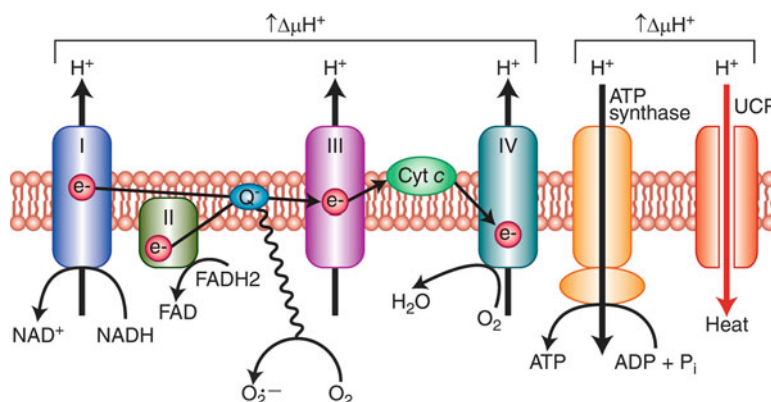


Fig. 2.3 Production of superoxide by the mitochondrial electron-transport chain. Hyperglycemia causes an increase in electron donors from the TCA cycle generating a high mitochondrial membrane potential by pumping protons across the mitochondrial inner membrane. This inhibits electron transport at complex III, increasing the

half-life of free-radical intermediates of coenzyme Q (ubiquinone), which reduce O_2 to superoxide [reprinted from Brownlee M. *Biochemistry and molecular cell biology of diabetic complications*. Nature. 2001;414(6865): 813–20. With permission from Nature Publishing Group]

inner-membrane-associated enzyme complexes, along with cytochrome C, and the mobile carrier Coenzyme Q. NADH has the greatest potential to generate ATP as it donates electrons to complex I, while $FADH_2$ donates electrons to complex II. Electrons from both of these complexes are subsequently passed to coenzyme Q, complex III, cytochrome C, complex IV, and finally to oxygen, which is then reduced to water. The voltage generated across the inner mitochondrial membrane drives the synthesis of ATP. Elevated glucose levels increases glycolysis and, thereby, enhances electron donation to the ETC. An increase in electron flux raises the voltage across the membrane and generates a higher membrane potential eventually reaching a threshold where transport at complex III is blocked, increasing electron donation to O_2 at coenzyme Q, generating ROS, specifically superoxide (O_2^-) (Fig. 2.3) [18, 20].

Reactive Oxygen Species

ROS promotes atherosclerosis by blocking eNOS synthase, further increasing the production of other ROS, especially superoxide anion (O_2^-), in endothelial cells and vascular smooth muscle cells [4]. Superoxide initially reacts with NO to

form peroxynitrite ($ONOO^-$), a potent oxidant that selectively inhibits prostacyclin (PGI_2) by nitrating and disrupting PGI_2 synthase's iron-thiolate center. PGI_2 inactivation causes the buildup of its precursor, prostaglandin endoperoxide (PGH_2), which induces vasoconstriction and endothelial dysfunction. In addition, PGH_2 promotes the conversion of PGI_2 to thromboxane A_2 (TxA_2) by TxA_2 synthase. Both of these events activate the thromboxane (TP) receptor causing platelet aggregation, as well as vascular smooth muscle cell activation, apoptosis, and expression of pro-inflammatory adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial-leukocyte adhesion molecule (ELAM-1).

Peroxyntitrite is also responsible for uncoupling eNOS by targeting its zinc tetrathiolate cluster. Its interaction with zinc releases it from the tetrahedral conformation and disulfide bonds then form between the two monomers. This disrupts the catalytic activities of eNOS, decreasing NO synthesis and increasing production of ROS. Tetrahydrobiopterin (BH4), an important factor in eNOS function, is also targeted and interrupted by peroxynitrite [21]. Superoxide also inactivates the glycolytic enzyme glyceraldehyde-3-phosphate

dehydrogenase (GAPDH), which induces vascular injury through four main pathways described below.

Protein Kinase C Pathway

Increased intracellular hyperglycemia causes de novo synthesis of diacylglycerol (DAG), an intracellular lipid messenger, through increased synthesis of the glycolytic intermediate glyceraldehyde-3-phosphate. DAG activates protein kinase C (PKC), a serine/threonine-related protein kinase, downstream in both endothelial and smooth muscle cells. There are multiple PKC isoforms; however, the most notable in the pathogenesis of diabetic atherosclerosis are PKC- β and PKC- γ .

Hyperglycemia-induced PKC upregulation increases endothelial cell permeability, depresses NO production, and increases production of vasoconstrictors, such as ET-1 and TxA₂. PKC also contributes to the hypercoagulable and pro-inflammatory state seen in DM by upregulating plasminogen activator inhibitor type 1 (PAI-1) and nuclear factor- κ B (NF- κ B), respectively, in endothelial and smooth muscle cells. PKC can increase ROS levels by activation of membrane-associated nicotinamide adenine dinucleotide phosphate (NAD(P)H)-dependent oxidases and by inhibition insulin-stimulated production of eNOS [18, 20, 22].

Hexosamine Pathway

Inhibition of GAPDH by superoxide also diverts the glycolytic upstream metabolite fructose-6-phosphate into the hexosamine pathway. Fructose-6-phosphate is converted by glutamine:fructose-6-phosphate amidotransferase (GFAT) to glucosamine-6-phosphate and subsequently to uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc), which is a precursor for proteoglycans, glycolipids, and glycoproteins [18, 20]. In hyperglycemia, UDP-GlcNAc serves as a substrate for protein O-GlcNAcylation. Post-translation O-GlcNAcylation and subsequent ubiquitination

and degradation of atheroprotective proteins in the vasculature, such as eNOS and A20, tip the balance toward heightened atherogenesis while increasing the transcription of proatherogenic proteins, such as thrombospondin-1 [14, 23]. O-GlcNAcylation of the transcription factor Sp1 increases its transactivation and the downstream expression of Sp1-dependent expression of both transforming growth factor- β (TGF β) and PAI-1, both of which contribute the development of vascular disease through basement membrane thickening and increased thrombosis [14, 18, 20, 22, 23].

Advanced Glycation Endproducts

AGEs are formed intracellularly in vascular endothelial and smooth muscle cells during hyperglycemia by nonenzymatic post-translational modification. The first step is glycation of intracellular proteins by both glucose and glucose-derived compounds. Methylglyoxal-derived AGE, the primary intracellular AGE induced by hyperglycemia, is formed by modification of glyceraldehyde-3-phosphate, which accumulates secondary to ROS inhibition of GAPDH. AGEs cause dysfunction by modification of intracellular and extracellular proteins, the latter of which may serve as ligands for transmembrane RAGE receptors of the immunoglobulin superfamily present on endothelial cells, smooth muscle cells, macrophages, and lymphocytes. Their upregulation results in generation of further ROS and a pro-inflammatory, procoagulable state through upregulation of NF- κ B, tissue factor, and VCAM-1 [18, 20, 24]. RAGE upregulation is seen in diabetic atherosclerotic plaques and infarcted cardiac tissue [24].

Polyol Pathway

Aldo-keto reductase reduces a variety of carbonyl compounds to their respective sugar alcohols or polyols. During hyperglycemia, glucose and glucose-derived compounds, such as glyceraldehyde 3-phosphate, are converted to sorbitol by

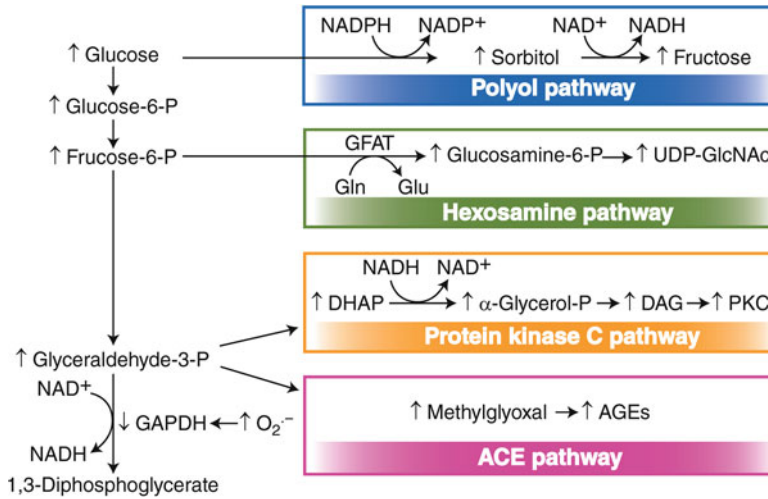


Fig. 2.4 Four pathways of hyperglycemia-induced injury through superoxide overproduction [reprinted from Brownlee M. *Biochemistry and molecular cell biology of*

diabetic complications. *Nature*. 2001;414(6865):813–20. With permission from Nature Publishing Group]

aldose reductase, which is then oxidized to fructose by sorbitol dehydrogenase (SDH) with NAD^+ as a cofactor. Increased ROS increases oxidative stress by consumption of NADPH, a cofactor required for regeneration of reduced glutathione (GSH) and an important scavenger of ROS. The subsequent conversion of sorbitol to fructose generates an additional NADH, further contributing to the overall oxidative state (Fig. 2.4) [18, 22].

Insulin Resistance

Obesity and a sedentary lifestyle are both predisposing factors for the development of insulin resistance and type II DM. In insulin resistance, there is an inadequate response by fat, muscle, and liver cells to insulin stimulation. Independent of hyperglycemia, insulin resistance is a risk factor for atherosclerosis. Insulin resistance in the type II diabetic patient is characterized by decreased insulin production, central stimulation for increased oral intake, increased gluconeogenesis in the liver, decreased uptake by peripheral tissues, and increased lipolysis of adipocytes leading to increased nonesterified fatty acid

(NEFA) secretion [25, 26]. NEFA can be deposited in and cause dysfunction of skeletal muscle, liver, and pancreatic β cells, all of which contributes to insulin resistance (Fig. 2.5).

Adipose tissue contributes to insulin resistance by releasing NEFA and pro-inflammatory cytokines, such as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1). Decreased skeletal muscle uptake of glucose is a side effect of insulin resistance and a significant contributor to hyperglycemia in type II DM. Increased intracellular NEFA in skeletal muscle is deleterious by competing with glucose for substrate oxidation and by increasing the intracellular content of fatty acid metabolites such as DAG, fatty acyl-coenzyme A (fatty acyl-CoA), and ceramide. In turn, these can activate a serine/threonine kinase cascade leading to serine/threonine phosphorylation of insulin receptor substrate-1 (IRS-1) and insulin receptor substrate-2 (IRS-2), reducing the ability of these receptors to undergo tyrosine phosphorylation and propagate the normal insulin signal. The downstream target of these receptors is phosphatidylinositol 3-kinase (PI3), which normally mediates insulin's physiologic anti-inflammatory signal by decreasing NF- κ b

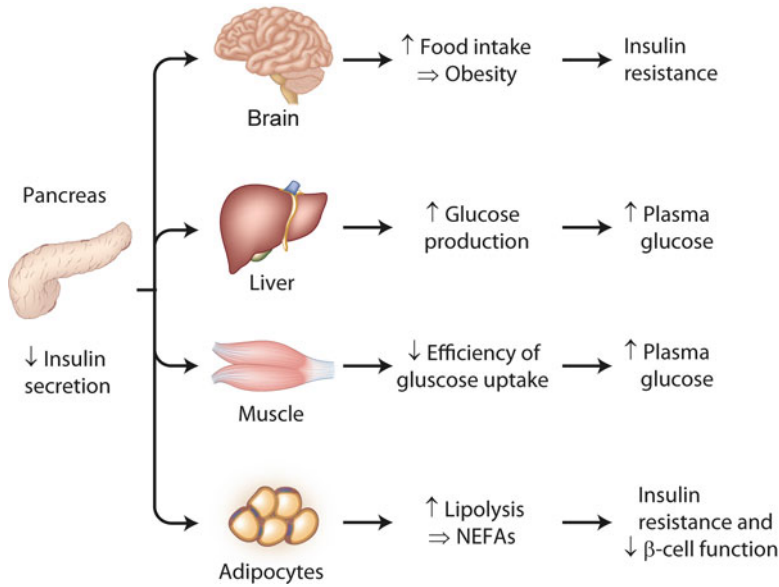


Fig. 2.5 Impaired insulin secretion results in decreased insulin levels and decreased signaling in the hypothalamus, leading to increased food intake and weight gain, decreased inhibition of hepatic glucose production, reduced efficiency of glucose uptake in muscle, and

increased lipolysis in the adipocyte, resulting in increased plasma NEFA levels [reprinted from Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. *Nature*. 2006;444(7121):875–80. With permission from Nature Publishing Group]

activation, ROS formation, expression of adhesion molecules, and increasing eNOS production [27–29]. PI3 blockade is also associated with decreased uptake of glucose and increased gluconeogenesis in the liver. DAG also has deleterious downstream effects through PKC production. The alternate pathway to PI3, mitogen-activated protein kinase (MAP-kinase), is hyperstimulated when the PI3 pathway is blocked by a compensatory increase in insulin production. This alters insulin's effect from anti-atherogenic to proatherogenic, as unopposed stimulation of the MAP-kinase pathway contributes to vascular hypertrophy, hypertension, increased PAI-1 production, and arrhythmias [28]. Events downstream of insulin-receptor signaling are diminished and the net effect is insulin resistance and diminished uptake of glucose (Fig. 2.6) [30].

The presence of increased adipose tissue is an important contributor to insulin resistance; however it is the distribution of this body fat that plays a key role in determining insulin sensitivity as intraabdominal visceral fat puts one at much higher risk. Even in lean individuals, body fat

distribution can markedly affect the degree of insulin resistance if there is increased visceral intraabdominal fat. Mesenteric fat, more than peripheral fat, interferes with insulin's ability to suppress lipolysis leading to higher NEFA production [31–33]. These mesenteric adipocytes tend to be larger and contribute to a pro-inflammatory environment in DM type II by increasing interferon- γ (IFN γ) expression, macrophage attraction, and upregulation of MCP-1 and NF- κ b [29, 34]. Differences in adipocytes, combined with the proximity of the liver to intraabdominal fat, result in greater exposure to NEFAs in liver than in peripheral tissues. In fact, the liver can be insulin resistant at a time when the peripheral tissues are not. Increased delivery of NEFA to the liver also increases gluconeogenesis, as well as production of very low-density lipoprotein (VLDL) [35].

β cells, which contain insulin, are also affected by insulin resistance as they are unable to fully compensate for impaired insulin uptake. β -cell dysfunction can exist in obese individuals with high central fat even in the presence of normoglycemia.

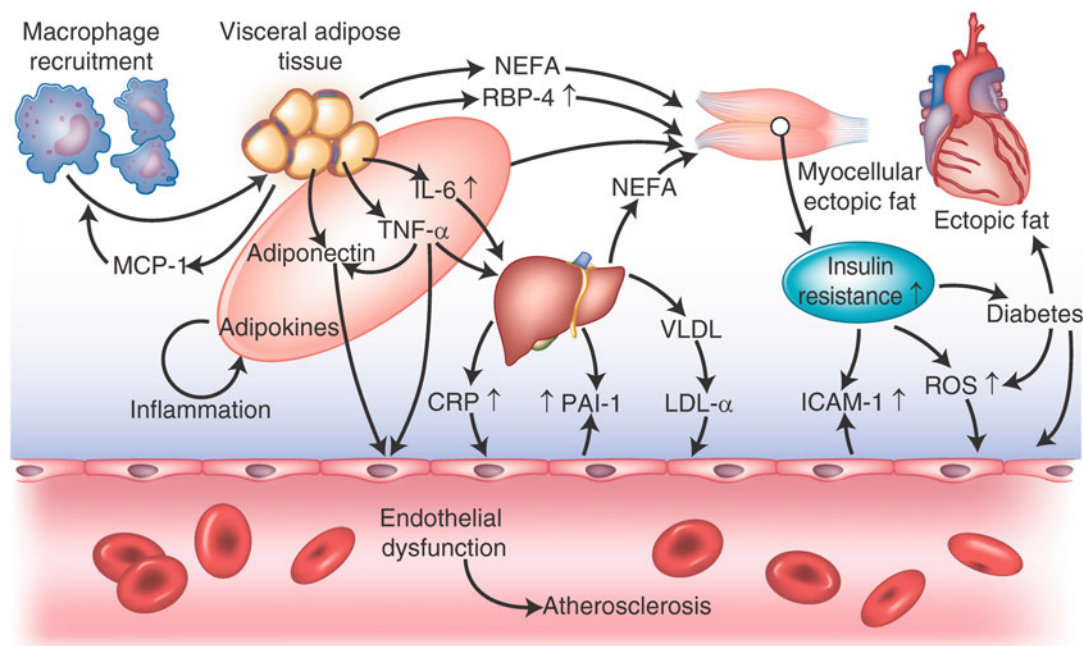


Fig. 2.6 Intraabdominal fat contributes to insulin resistance and cardiovascular dysfunction through cytokine (IL-6, TNF- α , and adiponectin), NEFA and retinol binding protein 4 (RBP-4) production [reprinted from Kahn

SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*. 2006;444(7121):840–6. With permission from Nature Publishing Group]

The β -cell is unable to produce insulin rapidly enough in response to high glucose levels. Insulin resistance further contributes to this effect by NEFA inhibiting insulin mRNA expression and insulin secretion. There are also half as many β cells in type II DM due to hyperglycemia-related toxicity. Impaired insulin secretion leads to decreased signaling in the hypothalamus, leading to increased food intake and weight gain, decreased inhibition of hepatic glucose production, reduced efficiency of glucose uptake in muscle, and increased lipolysis of mesenteric adipocytes with higher NEFA levels. An increase in body weight and NEFA production further contributes to insulin resistance [25].

Hyperlipidemia

The lipid profile generally seen in DM is one of elevated triglycerides, low high-density lipoproteins (HDL), and higher levels of small dense LDL particles. Increased fatty acid transport to

the liver stimulates the formation and secretion of VLDL [35]. The enzyme cholesterol ester transfer protein (CETP) catalyzes the transfer of triglycerides from VLDL to HDL in exchange for HDL cholesterol esters. These HDL enriched in triglycerides are an ideal substrate for hepatic lipase, whose activity is augmented in insulin-resistant states and type II DM, leading to increased breakdown of HDL particles. HDL levels are decreased and what HDL remains is functionally impaired, decreasing both its anti-inflammatory and antioxidant properties. Furthermore, glycation of apolipoprotein A-I (ApoA-I), a protein that is integral to the structure and function of HDL, disrupts the lipid–apoprotein interaction causing its disassociation from HDL. ApoA-I is renally excreted and the resultant HDL has reduced receptor binding activity [36–38].

In the presence of insulin resistance LDL levels are unchanged due to decreased production and catabolism. Their uptake is decreased because of a reduction in the number of LDL receptors

and their affinity for these receptors. LDL undergoes CETP-mediated exchange of VLDL triglycerides for LDL cholesterol esters. Hydrolysis of triglyceride-rich LDL generates small dense LDL particles that have increased affinity for LDL receptors, preferentially react with intimal proteoglycans, and are more likely to be taken up by macrophages to form foam cells. The proportion of oxidized LDL is also increased in DM due to glycation, increased triglyceride content, and the decreased anti-oxidative properties of HDL. Oxidative modification of LDL also results in rapid uptake by macrophages, leading to foam cell formation, increased cytokine production, and upregulation of cellular adhesion molecules in the endothelium, further contributing to inflammation and atherosclerosis [36, 38].

Endothelin-1

ET-1 is a potent vasoconstrictor that stimulates proliferation of smooth muscle cells and promotes fibrosis and inflammation leading to thrombosis and plaque formation in the vessel wall. ET-1 interacts with two distinct G-protein-coupled receptor subtypes—ET_A and ET_B. The upregulation of ET_A receptors and downregulation of ET_B receptors contribute to vascular dysfunction and atherosclerosis observed in DM [38]. ET_A receptors, which are localized mainly on smooth muscle cells and are responsible for vasoconstriction, proliferation, and ROS formation in response to ET-1. ET_B receptors are located on endothelial cells where they mediate vasodilation via the release of NO and prostacyclin (PGI₂). Plasma ET-1 levels are elevated in patients with both type I and type II DM. Elevated ET-1 levels have been linked to microalbuminuria and elevated glycosylated hemoglobin (HbA1c).

Platelet Aggregation

Increased platelet aggregation in DM is due to increased systemic production of isoprostanes, including TxA₂, increased responsiveness to platelet-activating factors (PAF) such as epinephrine

and ADP, and impaired production of PGI₂ and NO. DM also causes increased glycoprotein expression on the platelet surface, which increases platelet aggregation and platelet interaction with fibrin. Hyperglycemia can also upregulate PKC and generate ROS in platelets causing further dysfunction.

Whereas many of these pathophysiologic changes probably result from the metabolic consequences of insulin resistance, increased platelet reactivity has also been found in patients with type I DM without insulin resistance. Thus, hyperglycemia alone accounts for at least part of the altered platelet response due to AGE-related effects on platelet surface receptors [4, 39, 40].

Hypercoagulability

Independent of platelet dysfunction, diabetes induces a hypercoagulable state. Increased levels of PAI-1 decrease fibrinolytic activity and tissue factor, as well as factors VII and XIII are increased. There is also a relative decrease in antithrombin III and protein C. Many of these abnormalities also correlate with the presence of hyperglycemia and proinsulin split products. Von Willebrand's factor and factor VIII are also both increased, possibly due to endothelial dysfunction (Fig. 2.7) [4, 41].

Response to Injury

Endothelial progenitor cells (EPC) and vascular endothelial growth factor (VEGF) are important components in the vascular response to hypoxia and injury and are both impaired in DM.

Endothelial Progenitor Cells

Vascular injury and tissue ischemia trigger cytokine-mediated release of endothelial progenitor cells (EPC) from bone marrow into the peripheral circulation where they contribute to angiogenesis and repair areas of injured endothelium. Low EPC levels are generally associated

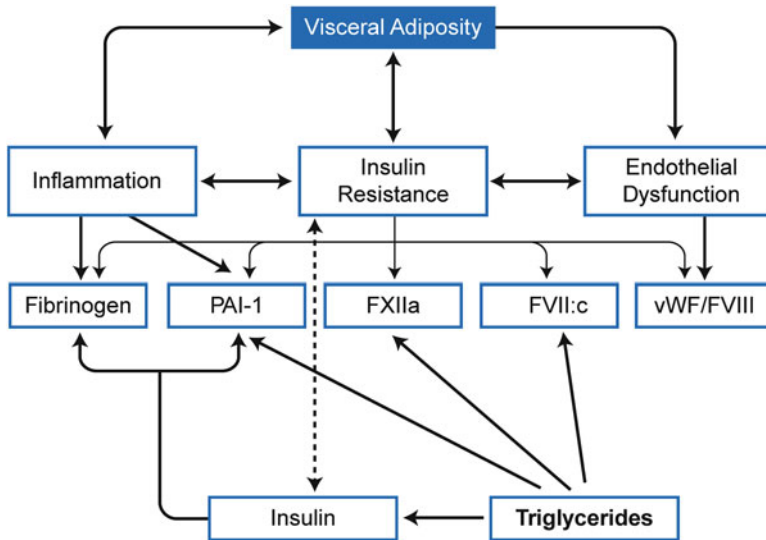


Fig. 2.7 Multifactorial causes of hypercoagulability in diabetes [reprinted from Grant PJ. Diabetes mellitus as a prothrombotic condition. *J Intern Med.* 2007;262(2):157–72. With permission from John Wiley & Sons, Inc.]

with higher cardiovascular complications and mortality. Tissue ischemia is considered to be the strongest stimulus for EPC release and occurs through activation of hypoxia-inducible pathways particularly upregulation of hypoxia inducible factor (HIF)-1. HIF-1 is a heterodimer consisting of two subunits that dimerizes in the nucleus under hypoxic conditions, which allows it to act as a transcription factor with cofactor p300. The glycolytic metabolite, methylglyoxal, can modify p300, forming an AGE, which decreases HIF-1-mediated gene transactivation. HIF-1 is also decreased due to ROS production and decreased NO. Decreased EPC levels in DM are thought to arise from decreased mobilization, proliferation, and survival as well as functional impairment (Fig. 2.8) [42–44].

Angiogenesis

A key feature of diabetes is poor collateral circulation. Angiography demonstrates fewer collateral

vessels in diabetic patients as compared to those without diabetes, which may contribute to a poor outcome after an acute occlusive event and difficulty healing lower extremity wounds. VEGF levels are diminished in the myocardium and lower extremity wounds in DM likely due to inhibition of HIF-1-mediated VEGF expression [20, 44].

Conclusions

Atherosclerosis is a major contributor to the morbidity and mortality observed in DM. The development of atherosclerosis is not only a result of hyperglycemia, but also from the secondary insulin resistance, dyslipidemia, hypercoagulability, altered secretion and function of local regulatory substances, and impaired response to injury. Strict glucose control alone is insufficient and a multifaceted approach targeting all mechanisms is required. A better understanding of these complex pathways is required to improve treatment and outcomes.

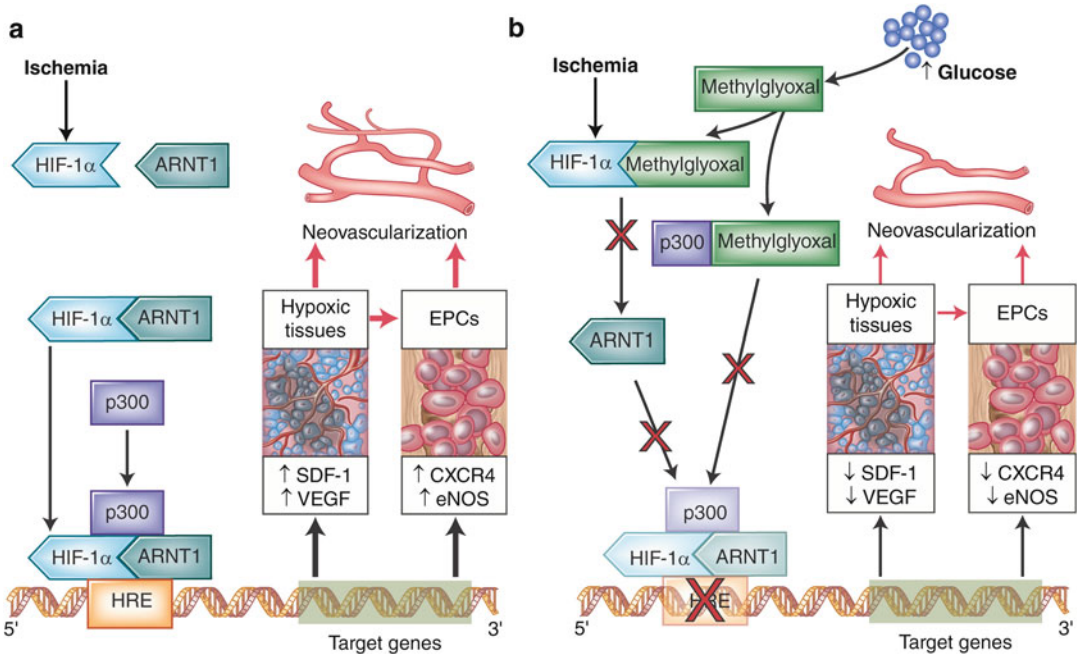


Fig. 2.8 Hyperglycemia inhibits HIF-1 upregulation of genes required for neovascularization [reprinted from Giacco F, Brownlee M. Oxidative stress and diabetic com-

plications. *Circ Res.* 2010;107(9):1058–70. With permission from Lippincott Williams & Wilkins]

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