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Addressing cardiovascular disease risk in diabetes: insights from mechanistic studies

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Abstract

Subjects with diabetes have increased cardiovascular disease risk compared to those without diabetes. Addressing residual cardiovascular disease risk in this disease, beyond blood pressure and LDL cholesterol control, remains important as the prevalence of diabetes increases worldwide. The accelerated atherosclerosis and cardiovascular disease in diabetes is likely multifactorial and there are numerous therapeutic approaches that can be considered. Results of mechanistic studies conducted in isolated cells, animals, or humans can provide important insights with potential to influence clinical management decisions and improve outcomes. In this review, we focus on three areas in which pathophysiologic considerations could be particularly informative in this regard; the roles of hyperglycemia, diabetic dyslipidemia (beyond LDL cholesterol level), and inflammation (including that in adipose tissue) for accelerating vascular injury and the rates of cardiovascular disease in Type 2 diabetes are outlined and evaluated.

INTRODUCTION

Multiple mechanisms likely contribute to the accelerated atherosclerosis and increased cardiovascular disease (CVD) risk observed in patients with Type 2 diabetes (T2DM). This review focuses on three areas in which basic mechanistic studies are most relevant to current clinical controversies for managing CVD risk in this disease. Pathophysiologic information relating hyperglycemia, diabetic dyslipidemia (beyond LDL cholesterol level), and inflammation to the accelerated vascular injury and CVD risk observed in T2DM will be evaluated and clinical considerations discussed.

HYPERGLYCEMIA AND THE VESSEL WALL

Although epidemiological studies show a consistent association between glycemic control and cardiovascular disease (1), the effect of tight glycemic control has been less convincing in

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Search Strategy Publicly available databases (PubMed, Medline) were searched. References were selected from peer-reviewed journals based on importance, novelty and relevance to the subject of the review.

clinical trials (2) The intensive glycemic control arm of the ACCORD (Action to Control Cardiovascular Risk in Diabetes) study was recently stopped due to increased cardiovascular deaths (3). A formal analysis of the study results has not yet been reported. The ADVANCE (Action in Diabetes and Vascular Disease) study will provide additional information regarding a potential CVD benefit of good glycemic control (4). Basic studies *in vitro*, in animal models, and in human subjects with diabetes suggest several mechanisms by which hyperglycemia might influence atherogenesis at the level of the artery wall (Fig 1).

Mechanisms by which hyperglycemia might affect vascular cells

Hyperglycemia could lead to vascular complications by several mechanisms. First, high glucose concentrations per se can activate nuclear factor κ-B (NFκB) (5;6), which in turn can increase the expression of a number of genes in endothelial cells, monocyte-macrophages and vascular smooth muscle cells. Advanced glycation end-products (AGEs) can be formed as a result of prolonged exposure of proteins and lipids to high concentration of glucose. AGEs include protein cross-links, fluorophors and other low molecular weight residues, which can generate reactive oxygen species. The ligation of AGEs to specific cell surface receptors can regulate gene expression in vessel wall cells.

Glucose also can increase oxidative stress, which in turn has several potential deleterious effects on the artery wall. For example, auto-oxidation of glucose leads to the formation of several reactive oxygen species such as the superoxide anion, and can facilitate LDL oxidation *in vitro* (7). Indirect evidence suggests that lipoprotein oxidation may be increased in diabetes *in vivo* (8) and is related to glycemic control (9). However, many of these studies relied on non-specific assays of oxidative stress. The absence of highly specific markers of oxidative stress in collagen (10) or in plasma or urine from subjects with diabetes (11) argues against a generalized increase in oxidative stress in diabetes. Thus, it has been suggested that glycoxidation reactions contribute to macrovascular disease in diabetes by damaging tissues in the local microenvironment of the arterial wall (11). Such pathways include the mitochondrial pathway for generation of superoxide, NADPH generation by monocyte-macrophages, or a redox-sensitive mechanism that generates hydroxyl radicals. The observation that products of hydroxyl radicals accumulate locally in arterial tissue of diabetic monkeys is consistent with the latter mechanism (12).

There has been a recent resurgence of interest in postprandial hyperglycemia as an important index of glycemic exposure and potential oxidative stress. 24-hour excretion of 8-iso prostaglandin $F_{2\alpha}$ (8-iso $PGF_{2\alpha}$), an indicator of free radical production derived from arachidonic acid in cell membranes (13), was increased in patients with diabetes compared nondiabetic controls (14). Among those with diabetes, 8-iso $PGF_{2\alpha}$ levels were highest in patients with the greatest glycemic variability. Moreover, glycemic variability was a strong predictor of total free radical production, whereas postprandial blood glucose levels were not. Indeed, swings in blood glucose levels accelerated atherosclerosis in apoE deficient mice (15). Additional studies are needed to determine the importance of oxidative stress that results from glycemic variability.

In the following sections we review experimental data of how glucose, AGEs and oxidative stress might influence atherogenesis. Available evidence will be reviewed for the three major types of vascular cells – endothelial cells, macrophages, and vascular smooth muscle cells (VSMC).

Glucose and the endothelium

An important initial event in the pathogenesis of atherosclerosis is the adhesion of monocytes to arterial endothelial cells, followed by their transmigration into the subendothelial space

along a chemotactic gradient. Hyperglycemia enhances monocyte adhesion to cultured aortic endothelial cells (16) by affecting both cell types. One mechanism involves activation of NF-κB by hyperglycemia (5;6). NF-κB activation increases the expression of several inflammatory genes, including adhesion molecules that facilitate monocyte adhesion to endothelial cells (5).

Adhesion molecule expression may also result from impaired nitric oxide (NO) production, since agents that increase the production of NO reduce the expression of these adhesion molecules. Glucose- and AGE-mediated inhibition of NO production by endothelial cells also is associated with impaired endothelial dependent relaxation (17-19), an early marker of vascular injury. In addition to displaying marked impairment of endothelium-dependent relaxation, diabetic mice have evidence of increased peroxynitrite generation, nitrotyrosine expression, and lipid peroxidation in aortic tissue (20). Hyperglycemia and AGEs also stimulate the production of superoxide by endothelial cells in part by activating NADPH oxidase (6;21), thereby providing a link between hyperglycemia, AGEs and oxidative stress.

Glucose and monocyte-macrophages

Both high glucose conditions (22-24) and AGEs are associated with an increased state of activation of circulating monocytes *in vitro* and *in vivo*. Monocytes grown in high glucose conditions or isolated from subjects with poorly controlled diabetes are in an activated and inflammatory state, as evidenced for example by increased expression of the cytokines, interleukin-1 beta and interleukin-6 (22), and expression of CD36 and monocyte chemoattractant protein-1 (25). These inflammatory changes are associated with induction of protein kinase C, NF-κB activation and increased release of superoxide, which could play a role in the oxidative stress that occurs in the presence of hyperglycemia (26).

Monocytes entering the endothelial space in response to chemotactic factors, proliferate and differentiate into intimal macrophages, which accumulate in the artery wall in diabetes (26) . Although hyperglycemia is not sufficient to stimulate macrophage proliferation in lesions of atherosclerosis or in isolated murine macrophages, a combination of hyperglycemia and hyperlipidemia stimulates macrophage proliferation by a pathway that may involve glucose-dependent oxidation of LDL (27).

Arterial wall macrophages can accumulate lipid from modified forms of LDL, which are taken up by scavenger receptors. These include LDL that have become oxidized as a result of glucose-mediated oxidative stress (28), and AGE-modified LDL (29). In addition, AGE-modified albumin can inhibit the selective uptake of cholesteryl esters from HDL (30), a critical step in reverse cholesterol transport. Thus, modification of lipoproteins and other proteins that result from prolonged exposure to high glucose conditions can alter the delivery and removal of lipid from macrophages in a manner that is likely to promote atherosclerosis.

Glucose and vascular smooth muscle cells

As lesions progress, smooth muscle cells migrate from the media to the intima, where they proliferate, generate growth factors, and participate in the formation of a fibrous cap. High glucose concentrations can stimulate the proliferation of VSMC *in vitro* (31). Similar finding are observed with exposure of cell to AGEs (32) and high insulin concentrations (33), which often accompany hyperglycemia in T2DM.

VSMC generate several matrix molecules that are involved in atherogenesis. Vascular proteoglycans bind atherogenic lipoproteins, leading to their retention in the subendothelial space (34). An increase in chondroitin sulfate and dermatan sulfate proteoglycans, and a decrease in heparan sulfate proteoglycans in atherosclerotic lesions at autopsy from subjects

with diabetes compared to lesions from nondiabetic subjects (35). The increase in chondroitin and dermatan sulfate proteoglycans in diabetes may contribute to the increased atherosclerosis in diabetes by increasing LDL retention in the artery wall (34). Diabetes also is associated with a loss of intimal elastin content and increased elastin fragmentation in both a rat (36) and pig (37) model of diabetes. Lower intimal elastin content, whether through decreased elastin production or increased elastin breakdown, appears to promote atherosclerosis by mechanisms that are not clear (37). Therefore, it is possible that elastin fragmentation is another mechanism by which hyperglycemia increases atherosclerosis in diabetes. Finally, collagen, which is made by VSMC, also accumulates in atherosclerosis. Advanced nonenzymatic glycation of collagen increases LDL binding, which might lead to increased lipoprotein retention by the artery wall in diabetes (38).

DIABETIC DYSLIPIDEMIA AND THE VESSEL WALL

Diabetic dyslipidemia is strongly related to atherosclerosis in human studies. Even though diabetic subjects may not have significantly elevated LDL cholesterol levels compared to matched subjects without diabetes, a cornerstone of managing CVD risk in diabetes rests on the use of LDL cholesterol-lowering statin drugs. Statin therapy generally reduces CVD events by 25-50% (39;40)but even among statin-treated subjects there appears to be excess residual CVD risk among those with diabetes compared to those without (41). Some of this residual risk could be attributed to lipoprotein abnormalities in diabetes that are not adequately addressed by statin therapy in T2DM. T2DM is characterized by decreased concentration of HDL cholesterol, increased concentration of triglyceride-rich lipoproteins, and abnormalities in the composition of HDL, LDL, and triglyceride-rich lipoprotein particles (Table 1) (42; 43). In this section we will evaluate mechanisms relating diabetic dyslipidemia to CVD risk. Available evidence will be reviewed organized by lipoprotein parameters, as these highlight the therapeutic questions that confront the clinician for managing cardiovascular risk in patients with diabetes.

Triglyceride-rich lipoproteins in diabetes

The triglyceride-rich lipoproteins in human diabetes are VLDL and its metabolites, and chylomicron remnants, and these can be elevated in the fasting or post-prandial state. The role of triglyceride-rich lipoproteins in human diabetic atherosclerosis remains controversial. Triglyceride levels vary inversely with HDL cholesterol levels, confounding interpretations relating elevations of triglyceride-rich lipoproteins to atherosclerosis (44). An interesting recent observation suggests that post-prandial triglyceride level may be a better predictor of CVD events than fasting triglyceride level independent of HDL cholesterol level (45;46). There is substantial *in vitro* support for a pro-atherogenic influence of triglyceride-rich lipoproteins in the vessel wall (Fig 2). Triglyceride-rich lipoproteins enhance a pro-inflammatory phenotype in endothelial cells and macrophages and produce apoptosis in endothelial cells (47). They increase tumor necrosis factor α (TNFα) expression in macrophages and increase expression of adhesion receptors resulting in increased adherence of monocytes and monocyte-derived macrophages to endothelial cells (48). ApoCIII, a component of triglyceride-rich lipoproteins and an inhibitor of lipoprotein lipase activity, increases adhesion of monocytic cells to endothelial cells (49). Chylomicron remnants and triglyceride-rich lipoproteins also produce lipid accumulation in macrophages (50). Uptake of larger lipid-rich VLDL particles is favored in macrophages, promoting lipid accumulation (51). Disruption of the VLDL receptor in macrophages reduces atherosclerosis in cholesterol-fed mice, while VLDL receptor expression in VLDL receptor-deficient recipient mice increases atherosclerosis (52). Lowering triglyceride-rich lipoproteins in a mouse model of diabetes and hyperlipidemia prevented disruption of atherosclerotic plaques (53). Elevated levels of post-prandial remnant lipoprotein particles have been shown to contribute to impaired arterial compliance (54).

The fatty acid composition of chylomicron remnants influences their uptake, and the induction of lipid accumulation in macrophages (50). The ability of triglyceride-rich lipoprotein particles to induce an inflammatory phenotype in macrophages may be enhanced by lipolytic release of fatty acids from VLDL (50;55). Elevated free fatty acid levels are also a component of diabetic dyslipidemia and accompany elevated triglyceride level. Fatty acids can directly lead to changes in the composition of extracellular matrix produced by arterial smooth muscle cells in a manner that favors increased immobilization and retention of lipoproteins (56). Excess free fatty acid delivery to peripheral tissues can worsen insulin resistance and may play a role in activating inflammatory processes through activation of toll-like receptors (57). Free fatty acids have also been shown to impair endothelium-dependent vasodilatation (58), and to disrupt the function of cellular sterol transporters important for reverse cholesterol transport (59), as further discussed below. On the other hand, there is data suggesting that in certain circumstances physiologic lipolysis of triglyceride-rich lipoproteins may have beneficial anti-inflammatory effects. In some model systems, the lipolytic release of fatty acids can provide a ligand for nuclear hormone receptors involved in repressing inflammation such as PPARγ (60;61). Taken together, these data suggest that inappropriate generation and/or handling of fatty acids may represent a fundamental abnormality in diabetes leading to accelerated atherosclerosis.

LDL in diabetes

Subjects with T2DM may not have significantly higher LDL cholesterol levels than matched subjects without diabetes, but for any LDL cholesterol level, those with diabetes generally have more LDL particles (42;44). This is because in T2DM, small dense lipid-poor LDL particles accumulate in the circulation. Because each LDL particle contains one apoB molecule, for any level of LDL cholesterol, subjects with T2DM will also have a higher level of apoB. An increased number of LDL particles, either directly measured or as indicated by apoB levels, may contribute to atherogenesis and CVD risk (62-64). Increased LDL particle number in diabetes can be therapeutically addressed by statin therapy. A separate issue has arisen, however, whether or not small dense LDL particles are inherently more atherogenic on a per particle basis, compared to larger buoyant particles. Evidence for increased atherogenicity of small dense LDL particles has been obtained from *in vitro* studies showing that small LDL particles rapidly enter the arterial wall, can be more toxic to endothelial cells, cause greater production of pro-coagulant factors, are oxidized more readily, and are more readily immobilized by proteoglycans present in the arterial wall (65). These particles have also been shown to bind less well to the LDL receptor, which may lead to impaired clearance by the liver (65). It remains unclear, however, how these *in vitro* results translate to the *in vivo* milieu. There is no completely satisfactory *in vivo* model for testing atherogenicity of small dense LDL particles on a per particle basis compared to larger particles and all apoB-containing lipoproteins with the exception of the largest chylomicrons can enter the subendothelial space and accumulate in the arterial wall. In non-human primates fed fat-modified diets, LDL particle size *per se* was not found to be independently atherogenic (66). Recent studies in humans found that both large and small LDL particles are related to atherosclerosis and CVD events (67; 68)

HDL in diabetes

Humans with T2DM have a decreased HDL cholesterol level and a decrease in the circulating concentration of apolipoprotein A1, the major apolipoprotein found in HDL (69). Abnormalities in size and composition of the HDL particle have also been noted (42;44;70; 71). HDL and apoA1 remove excess cholesterol from atherosclerotic plaque cells, and their reduced level in diabetes would be expected to have a detrimental effect on vessel wall cholesterol content (Fig 2). The cell type of most interest here is monocyte-derived macrophages as cholesterol ester engorged macrophages (*i.e.* foam cells) are hallmarks of the

atherosclerotic plaque. Removal of cholesterol from macrophages has been considered an important first step in the process of reverse cholesterol transport, and may be important for preventing progression or producing regression of atherosclerotic plaques (72). The HDL particle and its apoA1 component may act via distinct cellular sterol transporters for removing cholesterol from cells. The HDL particle appears to rely mainly on ATP binding cassette -G1 (ABCG1) to facilitate sterol efflux, and expression of ABCG1 in cells can be suppressed by exposure to glycosylated proteins (73). In addition, glycosylation of apoA1, which primarily acts via ABCA1, has been shown to suppress its ability to remove cholesterol from cells (74). HDL also has anti-inflammatory and anti-oxidant properties in cells of the vessel wall (75; 76) Monocyte-derived macrophages isolated from subjects with low HDL cholesterol manifest a pro-inflammatory phenotype (77).

In addition to changes to HDL cholesterol and apoA1 level, subjects with diabetes have altered HDL composition. HDL is perhaps the most heterogeneous and complex of all lipoprotein particles, and changes in composition may importantly impact HDL atheroprotective properties (78) (Fig 2). In isolated cells, HDL particles of different size and composition display different abilities to remove cholesterol from cells (79). Changes in content of numerous proteins associated with HDL, for example paroxonase, may impact its atheroprotective properties (80). Compositional abnormalities of HDL isolated from human diabetic subjects have been linked to impaired anti-atherogenic properties (70). The failure of cholesterol ester transfer protein inhibition with torcetrapib to protect against CVD events also underscores the notion that HDL particle composition may be more important than HDL cholesterol level for reducing CVD risk (81).

Mice with absent apoA1 and very low HDL cholesterol levels have more atherosclerosis because of both reduced cholesterol transport and increased inflammation (82). Conversely, increased expression of apoA1 with higher HDL cholesterol levels produces less atherosclerosis in the apoE-/- mouse – a model of accelerated and progressive atherosclerosis (83). An increase in HDL cholesterol in diabetic subjects has been linked to reduced carotid atherosclerosis (84;85). HDL has also been shown to improve mobilization and function of endothelial precursor cells (86); and to protect the myocardium from ischemia/re-perfusion injury (87).

Glycemia vs hyperlipidemia in the pathogenesis of atherosclerosis in diabetes

The relative roles of hyperglycemia and hyperlipidemia in atherogenesis have been difficult to separate in animal models of diabetes. Hyperlipidemia is usually exacerbated by the onset of hyperglycemia in mouse models such as LDL receptor and apoE deficient mice, thereby confounding the effect of hyperglycemia. However, two animal models suggest that hyperglycemia plays an independent role. First, fat-fed diabetic swine had more atherosclerosis than equally dyslipidemic fat-fed animals without diabetes (88). Second, consumption of a cholesterol-free diet by LDL receptor-deficient mice with a novel form of diabetes induced by a beta cell-directed viral antigen resulted in hyperglycemia without changes in lipids and lipoproteins (89). Hyperglycemia per se was associated with lesion initiation. Addition of increasing amounts of dietary cholesterol led to dyslipidemia, which was the major factor in atherosclerosis progression, independent of hyperglycemia (89).

CHRONIC SUB-CLINICAL INFLAMMATION AND THE VESSEL WALL

Extensive evidence ranging from human pathologic studies to *in vivo* mouse models establishes the role of inflammatory cells, like macrophages and T lymphocytes, and inflammatory mechanisms, like cytokine release in the pathogenesis of atherosclerosis (90). Several issues frame any consideration of the extensive pre-clinical data implicating inflammation in diabetic atherosclerosis. T2DM and atherosclerosis are both chronic conditions arising over decades,

a timeline that makes discerning cause and effect a challenge (Fig. 3). Inflammation is implicated in the pathogenesis of both T2DM and atherosclerosis (91;92). Since T2DM itself promotes atherosclerosis and increases cardiovascular events, a distinction may exist between inflammation that fosters T2DM versus inflammation that occurs subsequent to T2DM and that promotes atherosclerosis directly (Fig. 3). Most of the inflammatory mechanisms under discussion here also appear involved in the atherosclerosis seen in pre-diabetic as well as non-diabetic states. Here the focus is on those inflammatory mechanisms in diabetes that promote atherosclerosis and its complications, whether based in a given cell type or part of a more general pathway. Such an approach is required given that, although the evidence implicating inflammation in atherosclerosis and T2DM is extensive, no one specific mechanism, nor a single integrated framework, has emerged to explain precisely why patients with diabetes are more prone to inflammation or atherosclerosis.

Inflammation in Diabetic Atherosclerosis: Cellular Players

The endothelium, as the cellular interface between the circulation and the hyperglycemia and dyslipidemia that characterizes T2DM, responds to such stimuli by exhibiting an inflammatory response (93). Most of the responses induced in atherosclerosis are common to both diabetic and non-diabetic atherosclerosis. Classic pro-atherosclerotic endothelial responses – adhesion molecule expression, secretion of chemokines and coagulation proteins (plasminogen activator inhibitor 1, total plasminogen activator, tissue factor), release of vasoactive mediators (endothelial NO and bradykinin) – are induced or regulated by inflammatory stimuli in diabetes models *in vitro* and/or *in vivo* (94;95) as discussed earlier.

Lymphocytes, which provide critical pro-inflammatory signals to monocyte/macrophages, and VSMC, are activated by metabolic stimuli (96-98). Macrophages also directly respond to common abnormalities found in T2DM like glucose, free fatty acids and hypertriglyceridemia, by augmenting inflammatory responses (90;99). Multiple stimuli and cellular pathways are involved in these macrophage effects (Fig. 4), including increased foam cell formation, release of matrix metalloproteinases and secretion of growth factors and cytokines (92;100). Macrophages also highlight the important connections between insulin resistance, inflammation and atherosclerosis. When bone marrow from insulin receptor deficient mice was transplanted into LDL receptor deficient mice, more complex lesions were noted (101). Macrophage specific deficiency of the nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) in mice alters insulin resistance (102;103), suggesting that the presence of this ligand-activated transcription factor in macrophages regulates insulin sensitivity, an intriguing finding given PPARγ's role in repressing inflammation (104). Similar issues apply to retinoid signaling through the retinoid X receptor, the required partner for PPARγ and many other nuclear receptors (100;105).

Inflammation in Diabetic Atherosclerosis: Mechanisms

The available data suggests cellular responses to injury, inflammation and metabolism may converge on control points that are important in atherosclerosis. A central regulator of inflammation is NF-κB (106), a transcriptional complex activated by various stimuli including cytokines, oxidized LDL, lipopolysaccharide and oxidant stress (Fig. 4) (92). NF-κB reportedly regulates LDL oxidative modification, chemokine and cytokine expression, macrophage growth and differentiation, apoptosis, and VSMC proliferation. NF-κB, its regulatory proteins like inhibitor κB (IkB), and distal targets like the c-Jun n-terminal kinase (JNK) have all been strongly implicated in insulin sensitivity as well as atherosclerosis (92;100;107;108) (Fig. 4). As such, NF-kB may serve as a final common pathway linking many inputs that are activated in T2DM to atherosclerotic responses. NF-κB is activated by factors commonly abnormal in T2DM including fatty acids, glucose itself, AGE pathways, and certain toll like receptors (TLRs), a family of pattern recognition receptors found in various inflammatory cells (109).

The number of NF-κB regulated targets involved in diabetic atherosclerosis are extensive including TNF-α, which increases insulin resistance, TLRs, and resistin to name a few. In mice, inhibiting NF-κB activation can improve insulin sensitivity and decrease atherosclerosis, a concept under study in humans through several approaches, including high dose salicylates (92). PPARγ's anti-inflammatory and anti-atherosclerotic effects evident *in vitro* and in mice may also involve NF-κB inhibition (Fig 4) (104).

Several mechanistic pathways have been proposed for how glucose induces cellular injury and subsequent inflammation. A broad theme in this work suggests that cells lacking the ability to counter elevated intracellular glucose levels activate pathways of cellular injury and inflammation (see above) (110). These mechanisms include activation of protein kinase C, the formation of polyols, which promotes intracellular oxidative stress, and increased hexosamine activation, with subsequent increases in reactive oxidant species and mitochondrial stress (111-113). Although much of this evidence was linked to diabetic microvascular disease, increased flux of free fatty acids into the endothelium may influence macrovascular disease through similar pathways, inducing inflammation

All secretory and membrane proteins, many nutrients, and many pathogens pass through the endoplasmic reticulum (ER). Several lines of study implicate ER stress in promoting inflammation (114). Hypoxia, hyperglycemia, and increased fatty acids can all induce ER stress and a specific cellular process known as the unfolded protein response (UPR) (115), which is a homeostatic mechanism that restores normal ER function. ER stress, present in liver and in adipose tissue, can activate pro-oxidant and pro-inflammatory pathways and has been implicated in both diabetes and atherosclerosis (116).

Adipose tissue inflammation: A core mechanism in diabetic atherosclerosis?

Adipose tissue is now recognized as a biologically active endocrine and paracrine organ. This new view of fat has many implications for the intersection of inflammation, atherosclerosis and T2DM, especially given clinical data placing adiposity as a core defect in the metabolic abnormalities found before and during diabetes (94). Inflammation in adipose tissue itself may contribute to abnormal metabolism and atherosclerosis in T2DM.

Many of the pathways described above – oxidant stress, ER stress, NF-κB activation – also operate in adipocytes (94;100). Oxidative stress and inflammation in adipose tissue can be amplified by hyperglycemia (117). Fatty acids released from adipose tissue may signal to macrophages via pathways that involve toll-like receptors, leading to NF-κB activation. Interestingly, many of the same pathways involved in recruiting leukocytes to the arterial wall also recruit inflammatory cells to fat, including monocyte chemoattractant protein-1 (118). Indeed, mice lacking C chemokine receptor-2 (CCR2), the receptor for monocyte chemoattractant protein -1 (MCP-1), enjoy some degree of protection from diet-induced insulin resistance and induction of inflammatory programs (119). Excess lipid accumulation in other tissues, like skeletal muscle and the liver, may also modulate inflammation, contributing to insulin resistance and atherosclerosis (120;121).

Increased levels of inflammatory cytokines released from visceral fat in diabetes and obesity can act directly on the liver to increase the circulating levels of pro-inflammatory molecules such as C reactive protein (CRP) and serum amyloid A (SAA) (122). The former may directly amplify injury at the vessel wall and the latter unfavorably modifies the composition and function of HDL. The expression of adipose tissue apolipoproteins which impact adipocyte lipid metabolism, is also modified by inflammatory cytokines (123;124). Several adipocyte-specific mediators have been implicated in the inflammation contributing to insulin resistance and atherosclerosis. Leptin is an adipocyte-specific signal that appears to exert systemic pro-inflammatory effects (125). Leptin produces pro-inflammatory changes in endothelial cells

and macrophages, and administration of leptin to apoE-deficient mice promotes atherosclerosis (122). Adiponectin, which circulates in the plasma in various multimeric forms, limits inflammatory and atherosclerotic responses (126;127). Adiponectin levels are lower in obesity and diabetes (122;126) and the treatment of apoE-deficient mice with an adiponectin-expressing adenovirus has been shown to reduce atherosclerotic plaque formation (122). Adiponectin is present at higher levels in adipocytes from subcutaneous fat than visceral fat, one of many examples that suggest both depot-specific differences in fat and increased pathogenicity from visceral fat (122).

SUMMARY

Even in the statin era of lower LDL levels, hyperglycemia, lipoprotein abnormalities, and inflammation all appear intertwined in accelerating atherosclerosis and CVD risk in patients with diabetes. It is obvious from the data summarized here that important and complex links between hyperglycemia, lipoprotein abnormalities and systemic and vessel wall inflammation need to be considered when evaluating how each of these impact vessel wall health in diabetes. It is equally apparent that despite intensive study, persistent questions regarding these interactions require further investigation. Changes in glycemia impact lipoprotein levels, composition and metabolism, and can also directly impact inflammatory pathways, like NF-κB. Altered lipoprotein and lipid flux can modulate insulin sensitivity, glucose disposal, and thereby influence circulating glucose levels, as well as directly impact inflammation. Inflammation, both systemically and in adipose tissue, may play an important pathophysiologic role in overall insulin sensitivity and alter carbohydrate metabolism, lipid metabolism and inflammatory pathways in the liver, and directly add to vessel wall injury.

While pathophysiologic relationships do not always translate into high-value therapeutic targets, the abundance of pathophysiologic and mechanistic information from *in vitro* and animal models provides important insights for the design and interpretation of human clinical studies. These insights are also essential for understanding the implications of equivocal or negative results in randomized clinical trials. The recent failures of the intensive glucose control arm in the ACCORD trial (3), and of HDL cholesterol raising by the CETP inhibitor torcetrapib (81), to reduce cardiovascular death must be viewed in terms of the pathophysiologic and animal data related to these therapeutic areas. For example, data from *in vitro* and animal models would support the argument that the failure of intensive glycemic control in ACCORD should not eliminate the future consideration of hyperglycemia as an important therapeutic target for reducing CVD in diabetes, but may relate to an unfavorable benefit/risk ratio of currently available glucose-lowering therapies in the specific populations recruited for that trial. This elderly at-risk population may have been more susceptible to the adverse effects of hypoglycemia, off target drug effects, or the risk imposed by ectopic fat deposition that usually accompany intensification of glucose lowering therapy (128). With respect to the failure of torcetrapib, understanding the complexity of HDL metabolism and composition, and the potent atheroprotective effect HDL can have in animal models, argues that other methods of modifying HDL metabolism warrant evaluation. The lipid arm of ACCORD will provide information regarding the value of adding a fibrate (which can raise HDL cholesterol) to statin therapy in diabetic subjects (129). While therapeutic interventions in humans can never be as targeted or specific as the experimental manipulations achievable in isolated cells or in animal models, pathophysiologic and mechanistic information from these models remain key sources of insight for designing and evaluating new therapeutic options to reduce CVD in diabetes.

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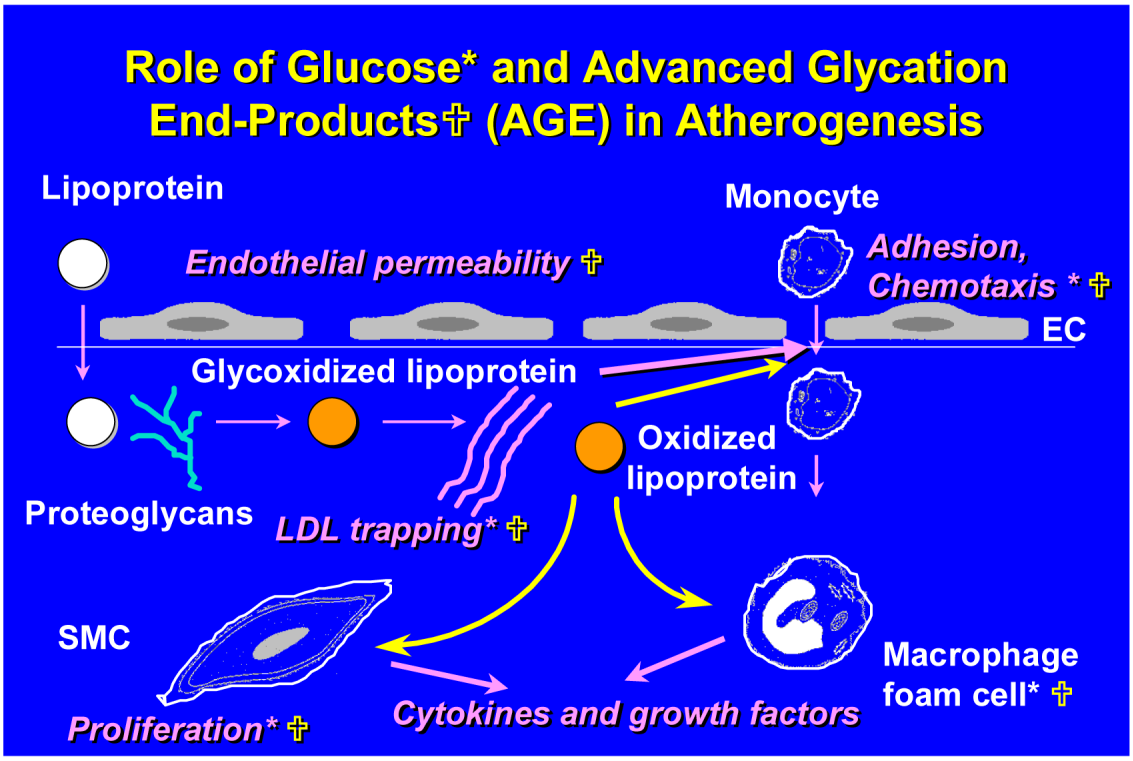

Figure 1. Potential ways in which glucose and advanced glycation end-products (AGE-proteins) can affect atherogenesis in diabetes

An early event in atherogenesis is adhesion of circulating monocytes to arterial endothelium. They enter the artery wall along a chemotactic gradient. Once inside the artery wall, monocytes can be activated and differentiate into macrophages. Atherogenic lipoproteins cross the endothelial barrier where they can be trapped by vascular proteoglycans and other matrix molecules such as collagen. Once retained by the matrix they can undergo modification by glycoxidation and other processes, which render the lipoproteins more toxic to vascular cells. They also can be taken up by macrophages, resulting in the formation of foam cells. Later, smooth muscle cell migrate from the media to the arterial intima. Glucose (*) and **AGEs** (✞) have been shown to affect various steps in these pathways as shown.

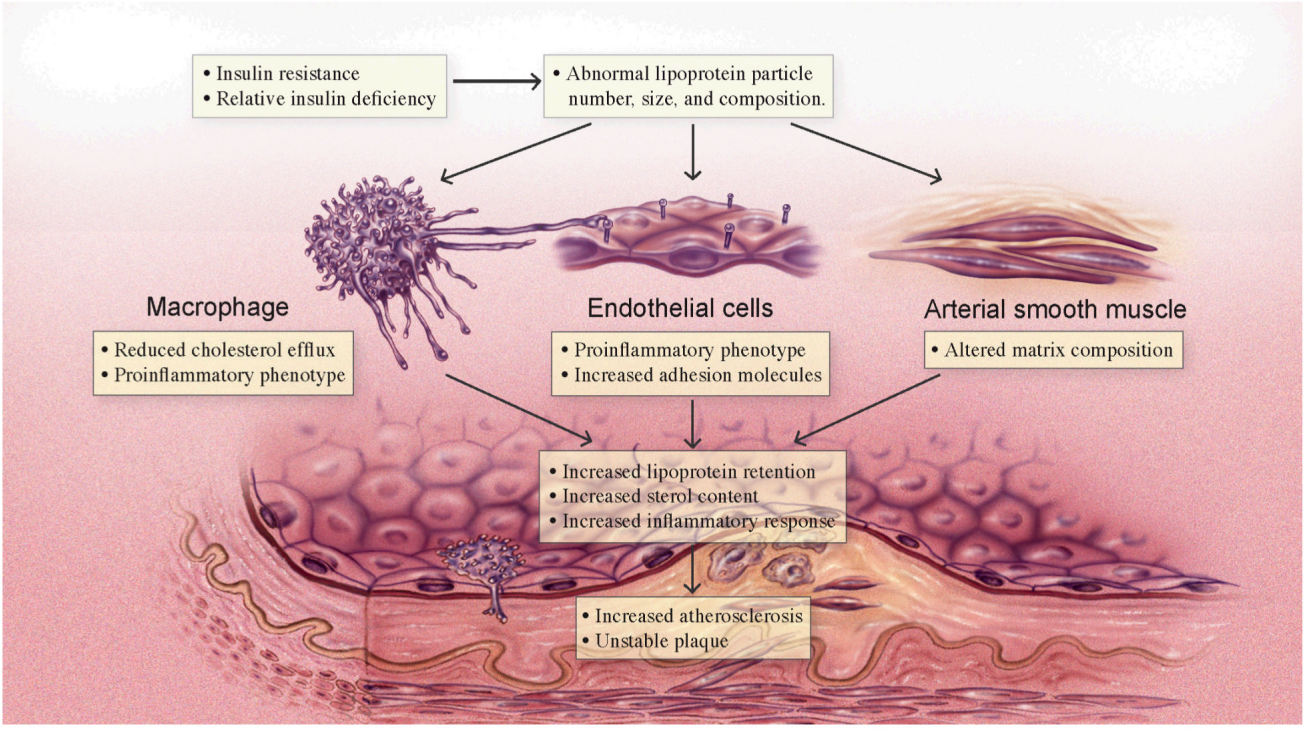

Figure 2. Diabetic dyslipidemia and the vessel wall

Insulin resistance and relative insulin deficiency in T2DM leads to abnormal lipoprotein particle number, size and composition. These lipoprotein changes impact gene expression and lipid flux in macrophages, endothelial cells, and arterial smooth cells to favor increased lipoprotein retention, sterol content, and inflammatory response in the vessel wall. These changes in the vessel wall favor growth of the atherosclerotic plaque and may predispose to instability and plaque rupture.

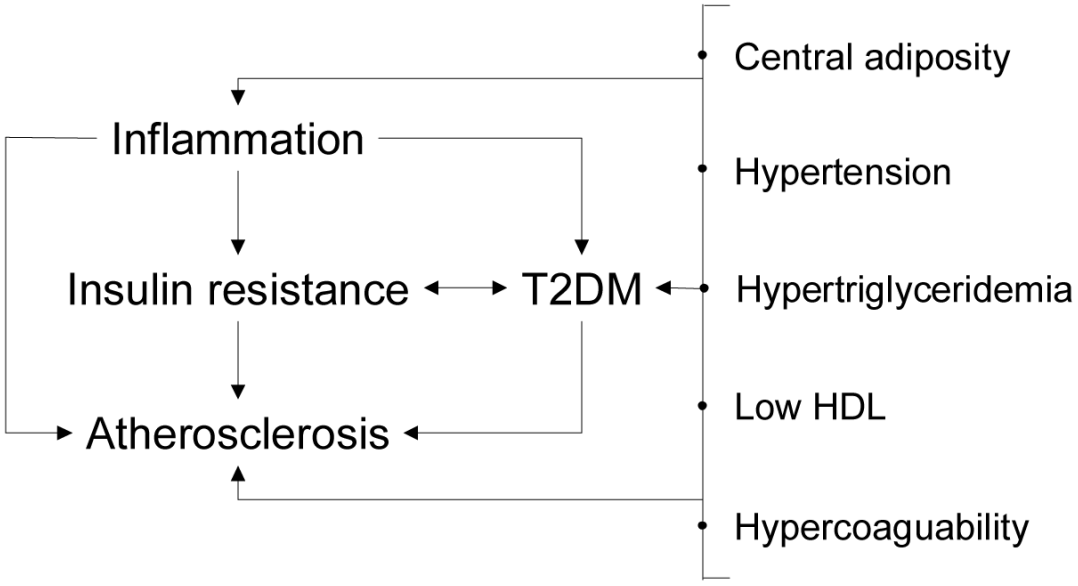

Figure 3. The intersection of inflammation, T2DM and atherosclerosis

Important relationships among inflammation, atherosclerosis, and key characteristics of T2DM are shown. Sorting out mechanistic relationships in humans is challenging given the chronicity of these problems, including relatively long pre-clinical phases and common, overlapping antecedents, like increased insulin resistance. For example, the constellation of abnormalities associated with T2DM, and pre-diabetic states, are also associated with cardiovascular risk. One common theme in these connections is inflammation.

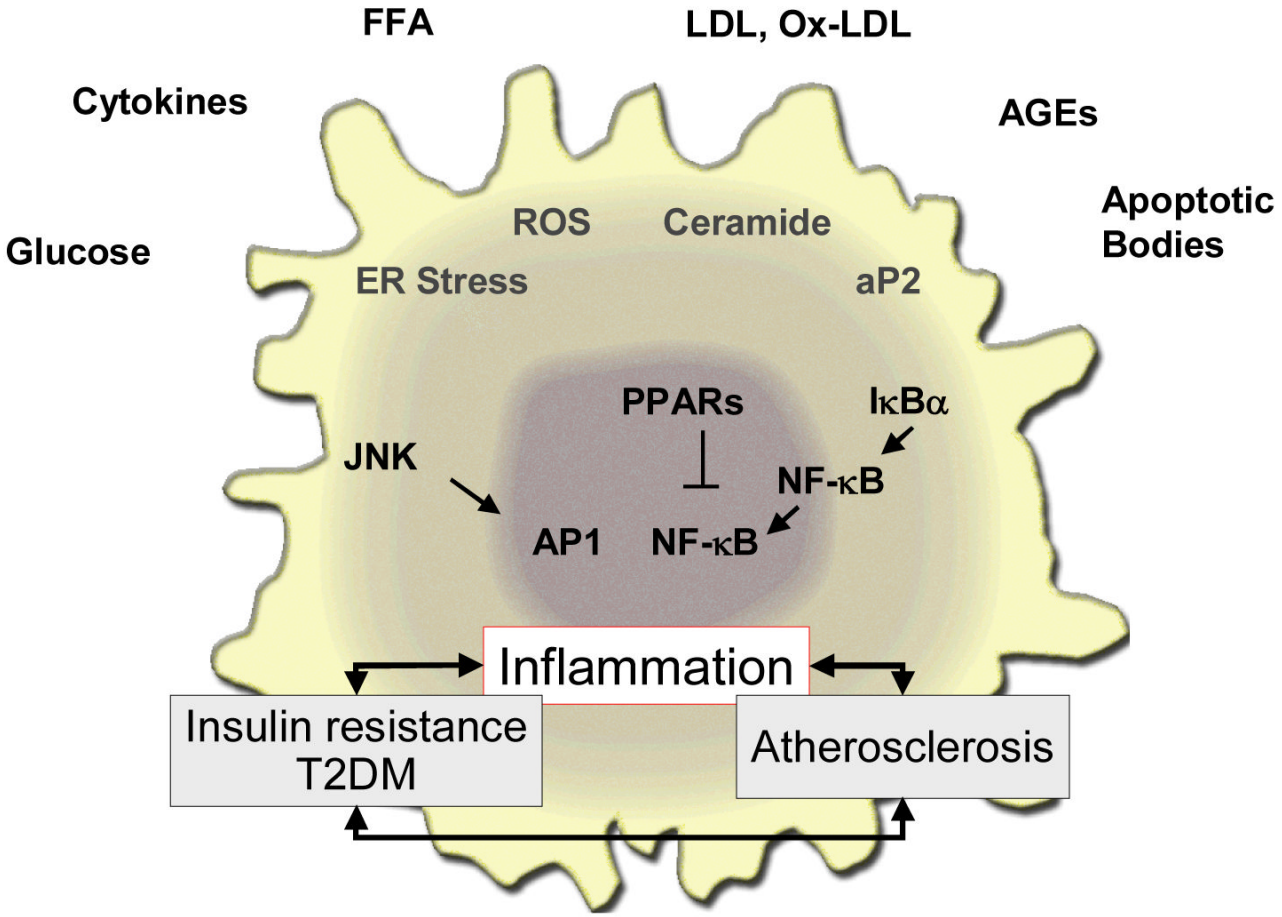

Figure 4. Macrophage biology in T2DM and atherosclerosis

The macrophage is a key player in atherosclerosis and may play an important role in the accelerated atherosclerosis of diabetes. Various factors commonly encountered in T2DM – hyperglycemia, elevated circulating cytokokines, increased free fatty acids (FFA), LDL and its modified forms, AGEs and cellular debris in the arterial wall (e.g. apoptotic bodies) can incite multiple responses in macrophages, including ER stress, generation of reactive oxygen species (ROS), and increased ceramide levels. These and other stressors can impinge on downstream inflammatory signaling pathways, such as JNK/AP1 and NFκB, further amplifying expression of a pro-inflammatory macrophage phenotype. Other mechanisms, like PPARγ activation, may balance some of these inflammatory responses.

Table 1

Lipoprotein Alterations in T2DM

Triglyceride-rich lipoproteins
Increased particle number
Increased post-prandial level
Triglyceride and cholesterol enriched particles
LDL
Increased particle number
Small dense particles
HDL
Decreased particle number
Numerous changes in particle composition

Patients with T2DM display characteristic abnormalities in lipoprotein level and composition.