Metabolic Karma—The Atherogenic Legacy of Diabetes: The 2017 Edwin Bierman Award Lecture

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Cardiovascular disease, despite all the recent advances in treatment of the various risk factors, remains the major cause of mortality in both type 1 and type 2 diabetes. Experimental models of diabetes-associated atherosclerosis, despite their limitations in recapitulating the human context, have assisted in the elucidation of molecular and cellular pathways implicated in the development and progression of macrovascular injury in diabetes. Our own studies have emphasized the role of oxidative stress and advanced glycation and identified potential targets for vasoprotective therapies in the setting of diabetes. Furthermore, it has been clearly shown that previous episodes of hyperglycemia play a key role in promoting end-organ injury in diabetes, as shown in clinical trials such as the UK Prospective Diabetes Study (UKPDS), Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation Observational Study (ADVANCE-ON), and the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC). The cause of this phenomenon, known as metabolic memory, remains to be elucidated, but it appears that epigenetic pathways, including glucose-induced histone methylation, play a central role. Further delineation of these pathways and their link to not only glucose but also other factors implicated in vascular injury should lead to more rational, potentially more effective therapies to retard diabetes-associated cardiovascular disease.

Cardiovascular (CV) disease remains the major cause of mortality and is associated with significant morbidity in both type 1 and type 2 diabetes (1–4). Despite major improvements in the management of traditional risk factors, including hypertension, dyslipidemia, and glycemic control prevention, retardation and reversal of atherosclerosis, as manifested clinically by myocardial infarction, stroke, and peripheral vascular disease, remain a major unmet need in the population with diabetes. For example, in the Steno-2 study and in its most recent report of the follow-up phase, at least a decade after cessation of the active treatment phase, there remained a high risk of death, primarily from CV disease despite aggressive control of the traditional risk factors, in this originally microalbuminuric population with type 2 diabetes (5,6). In a meta-analysis of major CV trials where aggressive glucose lowering was instituted, including Action to Control Cardiovascular Risk in Diabetes (ACCORD), Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation (ADVANCE), and Veterans Affairs Diabetes Trial (VADT), the beneficial effect of intensive glycemic control on CV disease was modest, at best (7). Nevertheless, the situation may be improving with the advent of some of the newer glucose-lowering agents.

Although numerous studies with dipeptidyl peptidase 4 inhibitors have demonstrated neutral effects on CV disease (8–10), recent trials with two sodium–glucose cotransporter 2 inhibitors, empagliflozin and canagliflozin (11,12), and two long-acting glucagon-like peptide 1 agonists, liraglutide and semaglutide (13,14), have reported CV benefits that have led in some of these trials to a decrease in CV and all-cause mortality. However, even with these recent positive CV outcomes, CV disease remains the major burden in the population with diabetes (15).

This unmet need of residual CV disease in the population with diabetes remains unexplained but may occur as a result of a range of nontraditional risk factors, including low-grade inflammation and enhanced thrombogenicity as a result of the diabetic milieu (16). Furthermore, a range of injurious pathways as a result of chronic hyperglycemia previously studied in vitro in endothelial cells (17) or in models of

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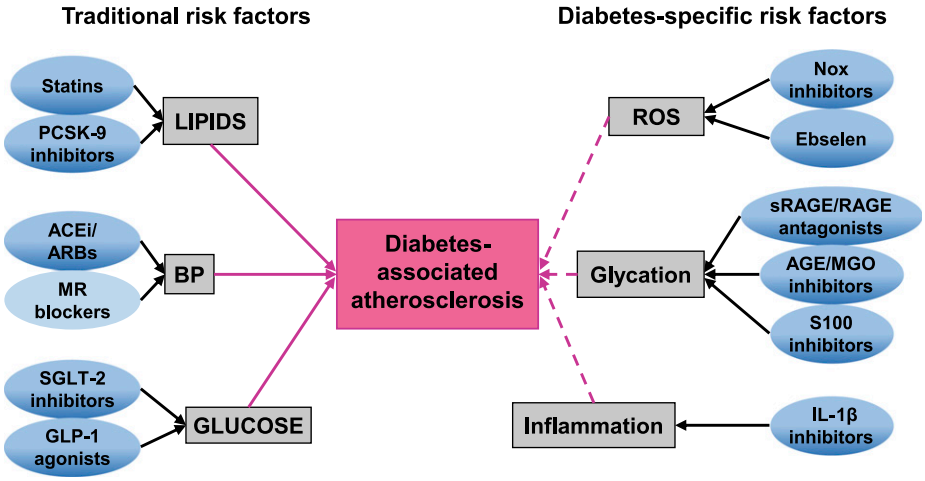

Figure 1—Traditional and diabetes-specific risk factors implicated in DAA are indentified and drugs that act to inhibit the deleterious vascular effects of these risk factors are listed. Magenta dotted lines represent potential as-yet not fully elucidated links of diabetes-specific risk factors to atherosclerosis, whereas the magenta solid lines reflect proven causation of certain traditional risk factors to macrovascular disease in diabetes. ACEi, ACE inhibitors; ARBs, angiotensin II receptor antagonists; BP, blood pressure; GLP-1, glucagon-like peptide 1; IL-1β, interleukin-1β; MR, mineralocorticoid receptor; PCSK-9, proprotein convertase subtilisin/kexin type 9; SGLT-2, sodium–glucose cotransporter 2.

microvascular complications may also be relevant and are a focus of this review (Fig. 1). One of the major stumbling blocks to studying the underlying molecular and cellular mechanisms responsible for diabetic macrovascular complications has been the lack of a suitable animal model. Twenty years ago, Schmidt and colleagues (18) induced diabetes with streptozocin (STZ) in the atherosclerosis-prone apolipoprotein E knockout (apoE$^{-/-}$) mouse and demonstrated within 6 weeks evidence of a diabetes-related increase in atherosclerosis in that model. Our group has subsequently performed numerous studies in this model including administration of various putative vasoprotective pharmacological agents and the generation of various knockout (KO) mice on the apoE$^{-/-}$ background (19–21). Although there are important limitations with these preclinical models, such as the absence of plaque rupture and circulating lipoproteins, which are different to that seen in man, the model demonstrated important pathological features that are seen in the human context, including diffuse atherosclerosis, prominent macrophage infiltration, and increased extracellular matrix accumulation. Furthermore, vasoprotective agents that have been shown to reduce atherosclerosis in man, such as agents that interrupt the renin-angiotensin system, have been shown to be end-organ protective in this model (19,20). The diabetic apoE$^{-/-}$mouse remains one of the preferred models by the JDRF/National Institutes of Health–sponsored Animal Models of Diabetic Complications Consortium (AMDCC) for the study of diabetes-associated CV disease (22).

ADVANCED GLYCATION PATHWAY

In the seminal study by Park et al. (18), soluble receptor for advanced glycation end products (sRAGE) was administered to diabetic mice and was shown to retard the development of atherosclerosis. This research built on a large body of research identifying the advanced glycation pathway and importantly a key receptor, RAGE, in the development of diabetes complications, albeit most of the previous research had focused on microvascular complications (23). Subsequent studies confirmed the importance of the advanced glycation end product (AGE)/RAGE axis by targeting this pathway using alternative approaches.

To complement the studies using sRAGE, which appears to act as a competitive antagonist inhibiting the binding of AGEs to full-length RAGE, studies were performed in RAGE KO mice on an apoE$^{-/-}$ background. These studies, initially performed in nondiabetic apoE$^{-/-}$ mice and subsequently in STZ-induced diabetic RAGE/apoE$^{-/-}$ mice, confirmed the importance of RAGE as a proatherogenic molecule (21,24). Complementary in vitro studies have clearly demonstrated a key role for RAGE, a receptor related to the Toll receptor family, in promoting vascular inflammation by enhancing expression of proinflammatory molecules and chemokines, at least in part by enhancing the generation of reactive oxygen species (ROS) (25) and activating the proinflammatory transcription factor NF-κB (26). Subsequent studies involving bone marrow transplantation have demonstrated key roles not only for bone marrow but also for endothelial-derived RAGE in promoting diabetes-associated atherosclerosis (DAA) (27). The importance of inflammation in CV disease has come from the recent positive findings in the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) trial where an interleukin-1β antagonist, canakinumab, was shown to confer CV protection in a population with previous CV disease; 40% of the subjects in this study had diabetes (28).

Pharmacological strategies to inhibit accumulation of this heterogeneous family of AGEs were explored, and two disparate approaches of preventive treatments to inhibit vascular AGE accumulation, aminoguanidine and the putative AGE cross-link

breaker alagebrium, reduced aortic plaque formations by >50% (29). In addition, as many patients with diabetes present clinically with atherosclerosis already present, our group administered AGE inhibitors in apoE$^{-/-}$ mice with established vascular disease. Using this delayed intervention approach, two AGE inhibitors, alagebrium and pyridoxamine, reduced vascular injury in the diabetic apoE$^{-/-}$ mice (30).

Although much of the focus has been on the role of AGE accumulation per se, it is increasingly appreciated that key dicarbonyl intermediates, such as methylglyoxal (MGO), which are important precursors in this pathway as a result of chronic hyperglycemia (31), may play a direct pathogenic role. To examine this possibility, our group adopted two different approaches to increase MGO levels in the absence of hyperglycemia (32). First, we fed animals MGO, and second, we administered an inhibitor of the enzyme glyoxalase-1 (GLO-1), which is responsible for MGO degradation. Both approaches not only resulted in elevated circulating MGO levels similar to those seen in diabetes but also were associated with an increase in plaque accumulation (32).

To further define the relative importance of AGEs, dicarbonyl intermediates, such as MGO and RAGE, have been used in RAGE KO mice in subsequent studies of DAA. MGO feeding enhanced atherosclerosis even in RAGE KO mice, suggesting that the proatherogenic effects of MGO are at least in part independent of RAGE (32). Previous studies by our group have shown that an AGE inhibitor reduced renal injury in diabetic RAGE/apoE$^{-/-}$mice (33), further indicating that RAGE is not essential to mediate the proatherogenic effect of AGEs or related precursors, such as MGO. Recently, we have confirmed a benefit of the AGE inhibitor alagebrium on atherosclerosis in this model, even in the absence of RAGE in association with a reduction in macrophage infiltration and oxidative stress, as reflected by vascular nitrotyrosine staining (Fig. 2). These findings emphasize the complexity of the AGE/RAGE interaction in mediating DAA, with evidence of a role for AGEs and precursors such as MGO, which can be generated by glucose and lipid peroxidation products (34), which are also elevated in subjects with diabetes. Furthermore, it appears that AGEs and dicarbonyl intermediates, such as MGO, can induce vascular inflammation independent of RAGE, although the identity of these other receptors are as yet unproven; however, Toll receptors and various scavenger receptors are likely candidates.

In addition, although AGEs are endogenous ligands for RAGE, there are other ligands including the S100 family of molecules that bind to this receptor (35). Naggareddy et al. (36) have identified a key role for S100 A8/A9 in promoting atherosclerosis by inhibiting resolution of plaques through the enhancement of monocyte production. These S100 ligands that appear to have derived from neutrophils act via their interaction with RAGE to promote thrombocytosis, which could itself be an important factor in enhancing CV risk in diabetes (37). Finally, increased glycation of lipoproteins as is seen in the diabetic milieu could also be proatherogenic. Advanced glycated LDL could be more atherogenic than native LDL (38), and glycation of HDL impairs the function of HDL to promote cholesterol efflux (39).

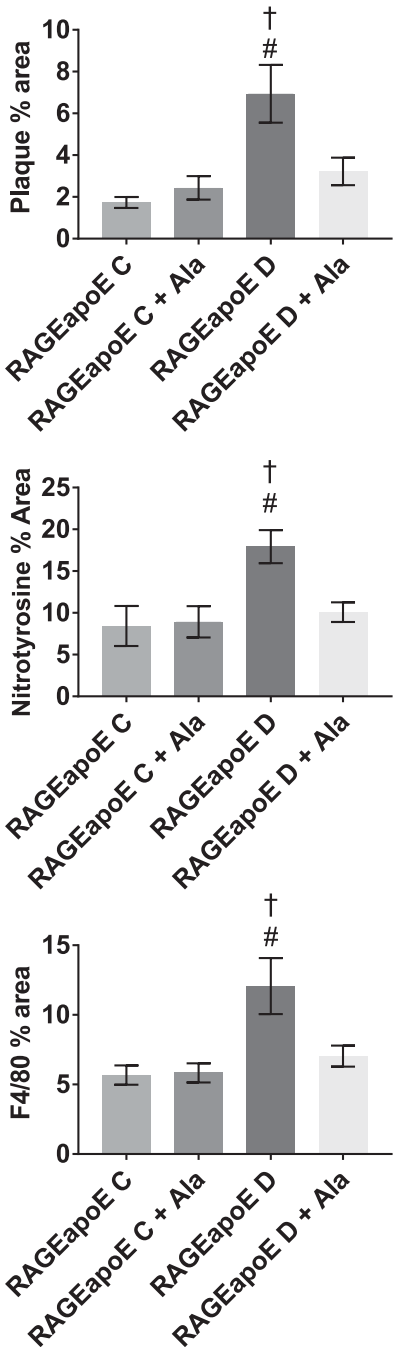

Figure 2—Data are shown as mean ± SEM for aortic plaque area (upper panel), vascular ROS accumulation as reflected by percent nitrotyrosine staining (middle panel), and the degree of macrophage infiltration as reflected by percent F4/80 staining (lower panel). Ala, alagebrium; RAGEapoE C, control RAGE/apoE$^{-/-}$mice; RAGEapoE D, diabetic RAGE/apoE$^{-/-}$mice. #$P < 0.05$ vs. RAGEapoE C; †$P < 0.05$ vs. RAGEapoE D + Ala.

OXIDATIVE STRESS

Another major factor that is likely to promote atherosclerosis in the diabetes setting is increased oxidative stress.

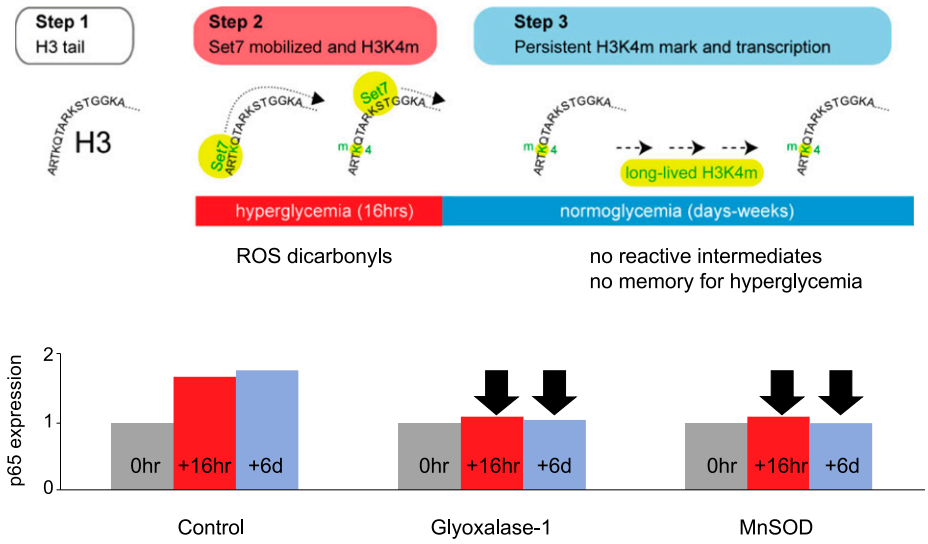

Figure 3—*Top*: Hypothesis implicating ROS and dicarbonyls in mediating glucose-induced Set7 mobilization to the tail of histone 3 (H3) (step 2), which leads to sustained H3K4m (step 3). *Bottom*: Gene expression of the p65 subunit in endothelial cells exposed to 16 h of high glucose (30 mmol/L, red column), followed by 6 days of return to normoglycemia (5 mmol/L, blue column) in the control setting and in cells transfected with GLO-1, an inhibitor of MGO accumulation, or MnSOD, an inhibitor of mitochondrial ROS. Both GLO-1 and MnSOD transfection abrogated the glucose-induced increase in p65 mRNA levels (arrows).

There is not only increased generation of ROS from diverse sources but also reduced antioxidant defense in diabetes (40). The increase in oxidative stress, as a result of hyperglycemia, was clearly demonstrated in the seminal studies by Brownlee and colleagues (17), initially performed in aortic endothelial cells. In these in vitro studies, mitochondrial sources of ROS generation were emphasized as a result of hyperglycemia.

Our own group has explored other sources of ROS generation with a focus on the enzyme family NADPH oxidase. This enzyme has multiple isoforms including Nox 1 and Nox 4. In the vasculature, Nox 1 is upregulated in diabetes and Nox 1 KO mice develop less atherosclerosis in the diabetes setting (41). Furthermore, GKT137831, an inhibitor of both the Nox 1 and Nox 4 isoforms, has been demonstrated to prevent atherosclerosis in diabetic apoE$^{-/-}$mice (41) and more recently was shown in a delayed intervention model to confer vascular benefits, including reduced plaque accumulation and less vascular inflammation in diabetic mice with established atherosclerosis (42). As this pharmacological approach inhibits not only Nox1 but also Nox 4, it was difficult to determine the specific mode of action of this drug. However, studies performed in Nox 4 KO mice indicate that in contrast to Nox 1, which promotes atherosclerosis (41), Nox 4 appears to be antiatherosclerotic. Nox 4 KO mice develop increased atherosclerosis in response to STZ-induced diabetes (43). Recent studies have identified a role for another Nox isoform, Nox 5, in vascular biology. As this isoform is not present in rodents, it has been more difficult to study. However, in vitro studies have suggested a role for Nox 5 in endothelial function (44). The role of this isoform remains to be determined in DAA, although limited studies using Nox 5 transgenic mice suggest a role for this isoform in diabetic nephropathy (45,46).

As outlined earlier, increased oxidative stress can also occur as a result of reduced antioxidant defense, which is indeed observed in diabetes. Our group has studied the impact of reduced antioxidant defense by performing experiments in mice deficient in the key antioxidant enzyme glutathione peroxidase 1 (Gpx1). STZ-induced diabetes in Gpx1/apoE$^{-/-}$ mice led to increased atherosclerosis, as reflected by increased aortic plaque, macrophage infiltration, and enhanced expression of various inflammatory markers (47). To complement these studies, a drug that repletes Gpx1, ebselen, was administered to diabetic mice, which led to reduced plaque accumulation (48). These findings demonstrating that vascular ROS accumulation is closely linked to atherosclerosis and vascular inflammation provide the impetus to consider specific antioxidant strategies as a novel therapeutic approach to decrease CV disease, particularly in the setting of diabetes.

EPIGENETIC PATHWAYS AND "METABOLIC MEMORY"

One of the most important findings from numerous trials performed in subjects with type 1 and type 2 diabetes has been the identification that previous episodes of hyperglycemia can have a long-standing impact on the subsequent development of CV disease. This phenomenon known as "metabolic memory" or the "legacy effect" has been reported in numerous trials, such as the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) (49) and the UK Prospective Diabetes Study (UKPDS) (50). The underlying explanation at a molecular and/or cellular level for this phenomenon remains to be determined.

Our group, as well as others, has postulated that epigenetic mechanisms may participate in conferring metabolic memory

(51–53). In in vitro studies initially performed in aortic endothelial cells, transient incubation of these cells in high glucose followed by subsequent return of these cells to a normoglycemic environment was associated with increased gene expression of the p65 subunit of NF-κB, NF-κB activation, and expression of NF-κB–dependent proteins, including MCP-1 and VCAM-1 (54).

In further defining a potential epigenetic mechanism that could explain the glucose-induced upregulation of genes implicated in vascular inflammation, a specific histone methylation mark was identified in the promoter region of the p65 gene (54). This histone 3 lysine 4 monomethylation (H3K4m1) occurred as a result of mobilization of the histone methyl transferase, Set7. Furthermore, knockdown of Set7 attenuated glucose-induced p65 upregulation and prevented the persistent upregulation of this gene despite these endothelial cells returning to a normoglycemic milieu (55). These findings, confirmed in animal models exposed to transient hyperglycemia (54), provide the rationale to consider Set7 as an appropriate target for end-organ protective therapies in diabetes. Although specific Set7 inhibitors are currently unavailable for clinical development, the current interest in drugs that block various enzymes, such as Set7, that influence histone methylation in diseases, such as cancer (56), could lead to agents that warrant testing in diabetes. Studies addressing other sites of histone methylation as well as other epigenetic pathways including DNA methylation and acetylation have been reported or are currently in progress (55,57,58), particularly in the context of diabetes complications. It remains to be determined if this research will lead to new targets to reduce the burden of diabetes complications, including macrovascular disease, or could potentially lead to novel biomarkers to predict complications and/or monitor disease progression and response to vasoprotective therapies.

As outlined earlier, it appears that certain carbonyl intermediates and ROS play a key role in linking hyperglycemia to vascular injury. Thus, our group assessed if glucose-induced changes in H3K4 methylation could be modulated by targeting these intermediates. In vitro studies showed that inhibitors of mitochondrial superoxide production and reduction in MGO by transfection of genes encoding the mitochondrial antioxidant manganese superoxide dismutase (MnSOD) and GLO-1, the major enzyme involved in MGO degradation, could abrogate the effects of glucose in mobilizing Set7, promoting H3K4m1 and activating NF-κB (54) (Fig. 3).

As in vitro and preclinical studies increase our knowledge and understanding of the pathogenesis of diabetes complications, it is likely that we will identify new molecular targets leading to better treatments to reduce the burden of macrovascular disease. Nevertheless, these new treatments will need to be considered in the context of improved management of traditional risk factors. This will include increased consideration of PCSK-9 inhibitors in at-risk patients with diabetes (59); better control of blood pressure including the potential use of newer agents, such as the mineralocorticoid antagonist finerenone (60); and newer classes of glucose-lowering drugs that afford end-organ protection, such as the sodium–glucose cotransporter 2 inhibitors (11,12) and certain long-acting glucagon-like peptide 1 agonists (13,14). It is anticipated that the burden of CV disease in diabetes will continue to decrease as has been recently reported (15) as more effective management of traditional risk factors is supplemented with newer agents that specifically target the key pathways that are primarily responsible for glucose-induced vascular injury and metabolic memory.

Duality of Interest. M.E.C. has received grants and honoraria from companies making drugs currently used for management of diabetes and its complications, including Boehringer Ingelheim, Lilly, Novo Nordisk, AstraZeneca, and Bayer. M.C.T. has received honoraria for educational symposia conducted on behalf of pharmaceutical companies involved in the management of diabetes and its complications, including Boehringer Ingelheim, Lilly, AstraZeneca, Novartis, Merck Sharp & Dohme, Sanofi, Servier, and Mylan. No other potential conflicts of interest relevant to this article were reported.

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