Do Glucose and Lipids Exert Independent Effects on Atherosclerotic Lesion Initiation or Progression to Advanced Plaques?

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Abstract—It is becoming increasingly clear that suboptimal blood glucose control results in adverse effects on large blood vessels, thereby accelerating atherosclerosis and cardiovascular disease, manifested as myocardial infarction, stroke, and peripheral vascular disease. Cardiovascular disease is accelerated by both type 1 and type 2 diabetes. In type 1 diabetes, hyperglycemia generally occurs in the absence of elevated blood lipid levels, whereas type 2 diabetes is frequently associated with dyslipidemia. In this review article, we discuss hyperglycemia versus hyperlipidemia as culprits in diabetes-accelerated atherosclerosis and cardiovascular disease, with emphasis on studies in mouse models and isolated vascular cells. Recent studies on LDL receptor–deficient mice that are hyperglycemic, but exhibit no marked dyslipidemia compared with nondiabetic controls, show that diabetes in the absence of diabetes-induced hyperlipidemia is associated with an accelerated formation of atherosclerotic lesions, similar to what is seen in fat-fed nondiabetic mice. These effects of diabetes are masked in severely dyslipidemic mice, suggesting that the effects of glucose and lipids on lesion initiation might be mediated by similar mechanisms. Recent evidence from isolated endothelial cells demonstrates that glucose and lipids can induce endothelial dysfunction through similar intracellular mechanisms. Analogous effects of glucose and lipids are also seen in macrophages. Furthermore, glucose exerts many of its cellular effects through lipid mediators. We propose that diabetes without associated dyslipidemia accelerates atherosclerosis by mechanisms that can also be activated by hyperlipidemia. (Circ Res. 2007;100:769-781.)

Key Words: Atherosclerosis ■ Diabetes ■ Fatty acids ■ Glucose ■ Mouse models
It is becoming increasingly clear that suboptimal blood glucose control may accelerate cardiovascular disease, at least in patients with type 1 diabetes. The Diabetes Control and Complications Trial (DCCT) involved young patients with type 1 diabetes assigned to conventional or intensive insulin therapy between 1983 and 1993. At the end of the DCCT, there had been few cardiovascular events, and there was no significant difference between the groups. However, the progression of carotid intima/media thickening, as a measure of atherosclerosis, was significantly reduced in patients on the intensive insulin regimen compared with patients on conventional therapy. Most of these patients were then followed in the Epidemiology of Diabetes Interventions and Complications study, during which glycemic control in the 2 groups was similar. Interestingly, intensive insulin therapy during the DCCT reduced the risk of cardiovascular events by 57% more than 10 years later. This effect was significantly associated with improved glycemic control during the DCCT phase of the study. The mechanisms underlying the long-lasting effects of improved blood glucose control are unclear. It is possible that hyperglycemia leads to long-lasting modifications of the arterial wall, for example, by stimulating advanced glycation endproduct (AGE) modification of proteins. It is also possible that increased lesion initiation results in more cardiovascular events many years later. The role of glycemic control in cardiovascular end points associated with type 2 diabetes is less clear, based on intervention studies. It is possible that in type 2 diabetes, dyslipidemia is more important as a factor driving cardiovascular disease.

This review article focuses on lipids versus glucose as cardiovascular risk factors associated with diabetes and discusses findings indicating that glucose may exert primarily "lipid-like" effects in vascular cells.

**Type 1 Diabetes Can Accelerate Lesion Initiation Through Mechanisms Independent of Diabetes-Induced Hypercholesterolemia**

Type 1 diabetes accelerates lesion initiation in several animal models and in humans. In some mouse and swine models, type 1 diabetes increases formation of lesions without associated increases in plasma lipid levels. However, in most mouse models, the extent of atherosclerosis in diabetic mice increases in association with a marked increase in plasma lipoproteins. Recently, we developed a mouse model that allows dissection of the relative contributions of diabetes versus diabetes-induced dyslipidemia. This model is a LDL receptor (LDLR)-deficient mouse (C57BL/6 background) that expresses a lymphocytic choriomeningitis virus (LCMV) glycoprotein (GP) transgene under control of the insulin promoter. The GP protein is seen as "self" and does not result in pathologies in the mouse. Instead, the significance of the expressed GP transgene is that diabetes can be induced at will by LCMV injection, which causes a T cell–mediated destruction of the GP-expressing β-cells, similar to the autoimmune process leading to type 1 diabetes in humans. Diabetic LDLR \(^{-/-}\);GP mice fed a semipurified low-fat diet do not develop marked diabetes-induced dyslipidemia but show accelerated lesion initiation compared with nondiabetic controls. Formation of these lesions is normalized by intensive insulin therapy following development of overt diabetes, showing that the effects on lesion initiation are caused by diabetes—most likely hyperglycemia in the setting of the basal hyperlipidemia in LDLR \(^{-/-}\) mice. Cross-sections of the brachiocephalic artery showed no lesions in nondiabetic mice, whereas fatty streak lesions were present in diabetic mice, as illustrated in Figure 1 (left). Lesions in the diabetic mice consisted primarily of macrophages. Further evidence in support of the hypothesis that hyperglycemia can stimulate lesion initiation comes from a recent study by Goldberg and colleagues, in which human aldose reductase transgenic mice were crossed with LDLR-deficient mice. Aldose reductase converts glucose to sorbitol, which is then further converted to fructose in the polyol pathway, implicated in many of the complications of diabetes. Interestingly, diabetic human aldose reductase transgenic LDLR-deficient mice showed larger lesions compared with diabetic controls, but aldose reductase has no effect in nondiabetic mice. The mechanism or cell type responsible for this effect is not yet known, but aortas from diabetic mice expressing human aldose reductase had increased mRNA levels of 2 markers of reactive oxygen species: glucose 6 phosphate dehydrogenase and glutathione peroxidase. The selective effect of the aldose reductase transgene in diabetic mice argues for a role of glucose conversion through the polyol pathway in diabetes-accelerated lesion initiation. However, in addition to acting on glucose, aldose reductase can also act on lipids. Thus, further studies are needed to definitively demonstrate...
that the accelerating effect of diabetes on lesion initiation is attributable to hyperglycemia.

There is also evidence from human postmortem studies that type 1 diabetes accelerates lesion initiation, defined as increased fatty streaks or intima/media thickness, in the absence of diabetes-induced dyslipidemia in youths and children.23–25

Together, these findings show that type 1 diabetes can accelerate lesion initiation in humans and animal models without an associated increase in blood lipid levels. Hyperglycemia may play a role in accelerating lesion initiation, at least within the normal to high plasma cholesterol levels seen in humans. However, animal studies have clearly shown that hyperglycemia alone is insufficient for lesion initiation when total cholesterol levels are very low (<3 mmol/L; 100 mg/dL).14,16–17 Thus, diabetes-induced lesion initiation requires low basal levels of circulating lipids.

**Severe Dyslipidemia Masks the Effects of Diabetes on Lesion Initiation**

In diabetic animals with severe dyslipidemia, lesion initiation is primarily driven by hyperlipidemia, which appears to overshadow the atherogenic effect of other risk factors, such as hyperglycemia or AGES.17,26 Thus, when plasma cholesterol levels are dramatically elevated (more than \(\approx 16\) mmol/L; 600 mg/dL), the effects of diabetes on lesion initiation are masked. We hypothesize that high lipid levels mask the effects of diabetes because glucose and lipids use similar mechanisms to induce lesion formation. Several Western-type diet-fed mouse models show no additional effect of diabetes beyond that of hyperlipidemia.19 In the work of Reaven et al, LDLR-deficient mice were fed a high-fat diet for 6 months.26 Although streptozotocin-diabetic LDLR\(^{-/-}\) mice were hyperglycemic and showed higher levels of AGES, they had similar plasma cholesterol levels as controls (both \(\approx 1000\) mg/dL) and did not demonstrate increased aortic lesion area. Furthermore, in the LDLR\(^{-/-}\):GP model, diabetes accelerates lesion initiation when the mice are fed a low-fat diet, but when fed a high-fat diet resulting in blood cholesterol levels greater than 1000 mg/dL, lesion initiation is driven by plasma lipid levels.17

Together, these studies indicate that severe hyperlipidemia overrides the effects of diabetes on lesion initiation. However, cholesterol levels greater than 600 mg/dL are rarely seen in humans, even in individuals with familial hypercholesterolemia, and although these findings are of scientific interest, their clinical significance is unclear. Importantly, lesion initiation does not necessarily result in clinical symptoms, and it has been suggested that macrophage-rich fatty streak lesions regress unless a fibrous cap of smooth muscle exists to encapsulate the macrophages.27 Many animal studies are restricted to these early lesions.

**Cellular Mechanisms Involved in Diabetes-Accelerated Lesion Initiation**

**Monocyte–Endothelial Adhesion**

It is generally believed that infiltration of monocytes into the subendothelial space, and subsequent accumulation of lipid-loaded macrophages, causes lesion initiation. In young human subjects, accumulation of lipid-loaded macrophages is often seen in areas with intimal smooth muscle–rich masses.27–28 In many small animal models, on the other hand, macrophage accumulation occurs in areas without preexisting intimal thickening. Recruitment of monocytes is regulated by endothelial adhesion molecules and their corresponding monocyte ligands. The most studied endothelial adhesion molecules include E-selectin, P-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intracellular adhesion molecule-1, but there are many more.29

Studies in animals and humans indicate that diabetes causes an increased endothelial expression of adhesion molecules. Thus, diabetic rabbits showed more endothelial VCAM-1 compared with nondiabetic rabbits.30 Hyperglycemia is likely to exert an acute stimulatory effect on endothelial adhesion molecule expression in vivo, because infusion of high (25 mmol/L) glucose leads to upregulation of endothelial P-selectin and intracellular adhesion molecule-1 immunoreactivity in rat mesenteric venules, accompanied by increased leukocyte adhesion.31 A study of a small group of human subjects with type 1 or type 2 diabetes showed increased endothelial VCAM-1 immunoreactivity compared with nondiabetic controls.32 Interestingly, RAGE (receptor for AGES) functions as an endothelial adhesion receptor by binding leukocyte \(\beta_{2}\) integrins.33 Expression of RAGE is increased in endothelial cells from patients with type 2 diabetes.34 Induction of RAGE might therefore serve as an additional pathway to increase monocyte–endothelial adhesion in diabetes.

In addition, monocytes from humans with poorly controlled type 1 or 2 diabetes exhibit increased adherence to endothelial monolayers,35 suggesting that a combination of increased endothelial adhesion molecule expression and increased ability of monocytes to adhere to these adhesion molecules contribute to the increased lesion initiation associated with diabetes.

**Subendothelial Macrophage Accumulation and Activation**

Endothelial adhesion of monocytes is required, but is not sufficient, for formation of macrophage-rich fatty streaks.36 Macrophage accumulation is increased in lesions from patients with type 1 and type 2 diabetes.37 A similarly increased macrophage accumulation has been observed in LDLR\(^{-/-}\):GP mice with and without diabetes-induced dyslipidemia.17,38

The effects of diabetes on lesion initiation is shown schematically in Figure 1 (left).

There is strong evidence that a mechanism whereby hyperlipidemia induces lesion initiation is through retention of lipoproteins by proteoglycans within the artery wall.39–40 Although diabetes does not consistently result in increased proteoglycan levels in the arterial wall, hyaluronic acid is increased in the media of arteries from patients with type 1 diabetes compared with controls.41
The LDLR\(^{-/-}\):GP mouse model shows similar increases in medial glycosaminoglycan accumulation in diabetic mice.17 This increased glycosaminoglycan staining is likely attributable, at least in part, to accumulation of
hyaluronic acid receptor CD44. Accordingly, mice monocyte binding through interaction with the monocyte of diabetes. Furthermore, hyaluronic acid can contribute to macrophage recruitment. Therefore, hyaluronic acid may contribute to diabetes.

Hyaluronic acid (a glycosaminoglycan) binds to the proteoglycan versican, which in turn can trap LDL. This is interesting because smooth muscle cell (SMC)-targeted overexpression of one of the hyaluronic acid–synthesizing enzymes (hyaluronic acid synthetase 2) causes lesion initiation in nondiabetic mice and thus mimics the effects of diabetes on advanced plaques. No animal or human studies have been reported in which direct effects of diabetes on advanced lesions were investigated without confounding effects on lesion initiation. It is therefore not known whether diabetes accelerates cardiovascular events merely by accelerating lesion initiation, or whether it has direct effects on the processes governing progression to advanced plaques.

Progression of Fibrous Cap and Necrotic Cores

Formation of a fibrous cap with proliferating SMCs is accelerated in diabetic swine, perhaps as a result of accelerated lesion initiation. Diabetic BALB/c mice show increased lesion initiation, which is also accompanied by SMC accumulation. Although formation of a fibrous cap plays an important role in early lesion progression, it is generally believed that thinning of this cap causes plaque rupture and clinical symptoms. It has recently been shown that forced SMC apoptosis in apolipoprotein E–null (apoE−/−) mice results in thinner fibrous caps and larger necrotic cores. It is not known whether diabetes specifically affects cap thinning in advanced plaques. On the contrary, apoptosis has been found to be reduced in SMCs derived from patients with diabetes and in SMCs exposed to elevated glucose.

It is clearer that diabetes can result in increased necrotic core formation (Figure 1, right). In postmortem studies, atherosclerotic lesions from patients with type 1 or type 2 diabetes were found to have larger necrotic cores than lesions from nondiabetic subjects. Necrotic cores form as macrophages undergo apoptosis or necrosis, possibly in combination with a decreased phagocytic capacity of surviving macrophages, resulting in accumulation of cell debris and free lipid. Accumulation of free unesterified cholesterol has been proposed to mediate macrophage death and thereby necrotic core formation. Diabetes has been associated with increased expression of receptors associated with uptake of modified LDL, such as CD36, scavenger receptor A, and lectin-like oxidized LDL receptor (LOX-1); and reduced levels of the reverse cholesterol transporters ATP-binding cassette A1 (ABCA1) and ABCG1, all of which could accelerate accumulation of intracellular cholesterol and potentially necrotic core formation. However, further studies are needed to investigate whether the ability of macro-

**Table**: Time Course of Changes in Brachiocephalic Artery Plaque Morphology in LDLR−/− Mice Fed Low- and High-Fat Semipurified Diets

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>12 Weeks</th>
<th>16 Weeks</th>
<th>30 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High-fat diet (1.25% cholesterol, 40% fat)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Cross-sectional area (×1000 mm²)</td>
<td>18±5*</td>
<td>146±35</td>
<td>171±25</td>
</tr>
<tr>
<td>Mac-2-positive area (×1000 mm²)</td>
<td>15±4*</td>
<td>16±2</td>
<td>14±3</td>
</tr>
<tr>
<td>Medial glycosaminoglycans</td>
<td>75±11</td>
<td>99±1</td>
<td>100±0</td>
</tr>
<tr>
<td>Intimal collagen</td>
<td>44±17</td>
<td>99±1</td>
<td>100±0</td>
</tr>
<tr>
<td>Necrotic core</td>
<td>24±16</td>
<td>77±7</td>
<td>100±0</td>
</tr>
<tr>
<td>Lateral macrophages</td>
<td>8±4</td>
<td>66±11</td>
<td>70±26</td>
</tr>
<tr>
<td>Intraplaque hemorrhage</td>
<td>0±0*</td>
<td>1±1</td>
<td>0±0</td>
</tr>
<tr>
<td>Cholesterol clefts</td>
<td>5±5</td>
<td>46±14</td>
<td>70±31</td>
</tr>
<tr>
<td>Intimal calcification</td>
<td>7±7</td>
<td>10±6</td>
<td>64±45</td>
</tr>
</tbody>
</table>

| **Low-fat diet (0% cholesterol, 11% fat)** |          |          |          |
| Cross-sectional area (×1000 mm²) | 1±1*     | ND       | 109±25   |
| Mac-2-positive area (×1000 mm²)  | 1±1*     | ND       | 9±2      |
| Medial glycosaminoglycans        | 26±8*    | ND       | 99±1     |
| Intimal collagen                 | 0±0      | ND       | 97±3     |
| Necrotic core                    | 0±0      | ND       | 74±11    |
| Lateral macrophages              | 0±0      | ND       | 77±11    |
| Intraplaque hemorrhage           | 0±0      | ND       | 1±1      |
| Cholesterol clefts               | 0±0      | ND       | 64±14    |
| Intimal calcification            | 0±0      | ND       | 30±10    |

Unless otherwise stated, results are shown as frequencies for lesion characteristics (mean±SEM percentage of brachiocephalic artery cross-sections scored positive for a given feature). The results are partly unpublished (F. Johansson, K.E. Bornfeldt, 2006) and partly summarized from Renard et al., Lamharzi et al., and MacDougall et al. *Processes stimulated by diabetes.*
phages in advanced atherosclerotic plaques to esterify free cholesterol to the less harmful cholesteryl ester is impaired, and whether this mechanism is affected by diabetes. It should be noted that macrophage apoptosis might affect early and advanced lesions differentially. Thus, reduced macrophage apoptosis results in increased formation of lesions in LDLR $^{-/-}$ mice. More relevant to type 2 diabetes are findings showing that macrophages lacking the insulin receptor (thereby being insulin resistant) are more susceptible to modified LDL-induced apoptosis and increased necrotic core formation in LDLR-deficient mice. This topic will be covered in detail in a forthcoming article in this thematic review series.

**Progression of Intraplaque Hemorrhage, Plaque Rupture, and Arterial Occlusion**

Cardiovascular events, most likely caused by plaque rupture, occur at an earlier age, and with an increased frequency, in patients with diabetes. Rupture of thin cap fibroatheromas appear to account for a majority of thrombotic events in patients with diabetes, as it does in patients without diabetes. In the mouse, rupture or fissuring of advanced plaques in the brachiocephalic artery and aorta is likely to result in intraplaque hemorrhage. Diabetes results in increased intraplaque hemorrhage, both in humans and fat-fed LDLR-deficient mice (Figure 1, right). In addition to disruption of the fibrous cap or the macrophage-rich shoulder regions, intraplaque erythrocytes can originate from leaking vasorum. In humans, intraplaque hemorrhage is often seen deep in the lesion and appears to result primarily from disrupted microvessels. Mice also develop increased networks of microvessels in response to atherosclerosis, at least in the aorta. In the smaller brachiocephalic artery, intraplaque hemorrhage is often seen in the plaque shoulder regions and most likely originates from the lumen as result of plaque disruption. It has been proposed that intraplaque hemorrhage can contribute to necrotic core formation by providing a large amount of cholesterol derived from erythrocyte cell membranes.

The mechanism whereby diabetes promotes intraplaque hemorrhage is unknown. However, it is interesting to speculate that diabetes might increase protease activity, which in turn would destabilize the fibrous cap or extracellular matrix surrounding the macrophage-rich shoulder regions. Recent studies in apoE$^{-/-}$ mice demonstrate that macrophage-targeted expression of matrix metalloproteinase 9 (MMP-9), or an active form of MMP-9, causes intraplaque hemorrhage. A recent study identified genes differentially expressed in stable and unstable areas of human advanced lesions. Several proteases were upregulated in unstable areas compared with stable areas from the same lesion, including MMP-9. Aortas from diabetic rats and internal mammary artery from patients with type 2 diabetes exhibit increased MMP-9 activity. Monocytes from patients with type 1 diabetes also show higher expression of MMP-9 than those of control subjects. Furthermore, advanced atherosclerotic plaques from patients with diabetes show increased MMP-9 expression. It is possible that hyperglycemia is directly responsible for the increased MMP-9 activity, as elevated glucose can stimulate MMP-9 expression in human macrophages and endothelial cells. Together, these studies suggest that the increased intraplaque hemorrhage associated with diabetes could be attributable to an increased activity of proteases, such as MMP-9.

Whereas many of the features of lesion initiation and progression discussed above can be addressed using mouse models, currently there are no ideal mouse models available for studies of arterial occlusion caused by thrombosis. Thus, whereas advanced atherosclerotic plaques in LDLR-deficient and apoE-deficient mice show many of the signs of advanced human lesions, and even undergo plaque rupture, thrombotic occlusion and cardiovascular events are extremely rare in the mouse. Clearly, better animal models are required for mechanistic studies addressing the effects of diabetes on atherothrombosis.

**Progression of Intimal Calcification**

Contrary to calcification of the medial layer, calcification of advanced lesions does not appear to be stimulated by diabetes in humans. Based on results from LDLR$^{-/-}$ mice, intimal calcification occurs late in lesion progression (Table). Thus, there is no evidence that intimal calcification is directly stimulated by diabetes in lesions of a similar stage as those of nondiabetic controls.

**Genetic Factors Regulating Effects of Glucose and Lipids on Lesion Initiation and Progression to Advanced Plaques**

It is clear from epidemiological and family studies that cardiovascular disease associated with type 2 diabetes has a genetic basis. However, the identification of specific genetic factors controlling cardiovascular disease in diabetes has been a daunting task. This is because both atherosclerosis and type 2 diabetes are multigenic disorders with contributions from environmental factors. In addition, each disorder proceeds through stages, the genetic components of which may vary in importance during disease evolution. Finally, the regulation of lipid and glucose homeostases are intertwined, making it difficult to separate influences of lipid and glucose metabolism on lesion initiation and progression. The discovery of specific genes involved in cardiovascular disease associated with diabetes is being performed in humans and mice using candidate gene and whole genome scanning approaches.

**Candidate Genes**

Association studies have been performed to examine relationships between gene polymorphisms and incidence or severity of sub-clinical and advanced atherosclerosis among individuals with and without insulin resistance or type 2 diabetes. Lipid metabolism genes already known to predispose to atherosclerosis, and glucose metabolism genes known to predispose to insulin resistance and diabetes, have provided the initial list of candidate genes. Increased prevalence and severity of cardiovascular diseases were seen among type 2 diabetic individuals with specific alleles of lipoprotein lipase, cholesteryl ester transfer protein, apoA-IV, apoA-II, and others. More recently, candidate gene studies have been performed to examine the effects of genetic variation in genes encoding regulatory molecules of cell function (e.g., chemokines, adhesion molecules), cell adhesion molecules, and matrix metalloproteinases on atherosclerosis. The results of these studies are consistent with a role for genetic variation in these genes in the development of atherosclerosis.
and peroxisome proliferator-activated receptor (PPAR)-α, with protection seen for an allele of PPAR-γ2.

Interestingly, few genes directly related to glucose metabolism have been connected to cardiovascular disease associated with diabetes. In one case, a common polymorphism controlling the basal and insulin stimulated expression of phosphoenolpyruvate carboxykinase was associated with carotid intimal media thickness in a small study of a Canadian Oji-Cree population. In another case, single nucleotide polymorphisms identified for tyrosine phosphatase-1B, which acts as a negative regulator of insulin and leptin signaling, associated with scores for coronary calcified plaques among a group of type 2 diabetic subjects.

Glycoxidation and lipid oxidation are thought to contribute to atherosclerosis and, therefore, it may be expected that alleles for genes that protect cells from oxidation damage may retard cardiovascular disease associated with diabetes. Common variants in the paraoxonase 1 gene were shown to modulate risk of coronary heart disease among subjects with type 2 diabetes. The important contribution to atherosclerosis of RAGE has been shown in several studies of diabetic and nondiabetic mice. Functional polymorphisms have been identified in the promoter of the human gene coding for RAGE, and the −374-AA genotype has been associated with protection against several diabetic complications. Studies of aldose reductase genotype variations in association with intima media thickness would be worthwhile, given the recent observation that elevations of aldose reductase can promote atherosclerosis in diabetic mice.

Mouse models have also contributed to our knowledge about genetic factors controlling accelerated atherosclerosis as a function of plasma glucose levels. For instance, simply deleting the LDLR gene ensures responsiveness to diabetes with respect to accelerating atherosclerosis as compared with wild-type LDLR knockouts. As compared with lack of accelerated atherosclerosis in wild-type C57BL/6 mice, Deficiency in lipocalin-type prostaglandin D2 synthase results in increased insulin resistance and formation of accelerated early atherosclerosis. Furthermore, the combination of a human apoB transgene and lipoprotein lipase deficiency with streptozotocin-induced diabetes results in hyperlipidemia and more atherosclerosis. These studies point to several potential candidate genes involved in diabetes-accelerated atherosclerosis.

An important consideration in the mouse is the role of background strain. In most cases, diabetes-accelerated atherosclerosis has been studied in genetically engineered mutants of C57BL/6. Although C57BL/6 mice are susceptible to atherosclerosis, as induced by special diets and gene mutations, compared with most strains, accompanying increases in plasma lipids characteristic of this strain may obscure subsequent insults to the vasculature resulting from hyperglycemia. It may be more useful to study mice more resistant to atherosclerosis, such as BALB/c, DBA/2, and NOD. For instance, diabetic BALB/c, but not C57BL/6, mice show increased lesion formation compared with their nondiabetic littermates. Furthermore, C57BL/6 mice carrying obesity/diabetes mutations fail to exhibit increased lesion formation as compared with wild-type controls. Thus, it is crucial that studies of diabetic complications are conducted in multiple genetic backgrounds.

Overall, only a few genetic studies in humans implicate specific lipid and glucose homeostasis genes in cardiovascular disease associated with type 2 diabetes. Such studies are limited by sample size and the strength of gene effects on vascular disease as seen in the diabetic state. However, the combination of studies demonstrates that specific genetic factors are important in determining risk for cardiovascular disease associated with type 2 diabetes. To date, there are a paucity of studies of the association between glucose and lipid homeostasis genes and cardiovascular disease associated with type 1 diabetes.

**Genome Scanning**

The work outlined above argues that diabetes-accelerated atherosclerosis is a multigenic disorder influenced by distinct contributions from many genes. Genome-scanning approaches may allow the simultaneous discovery of multiple loci responsible for atherosclerosis in human subjects or animals with diabetes.

Although many genome-scanning studies in humans have been applied to glucose homeostasis, obesity, insulin resistance, and diabetes, few have included quantitative traits associated with cardiovascular disease. The calcium-activated neutral protease 10 gene located on chromosome 2p was among the first genes linked to type 2 diabetes susceptibility using genome scanning. In this population of Mexican Americans, association of variation in calcium-activated neutral protease 10 was predictive for carotid intima/media thickness and measures of insulin resistance. In a recent study of families, which included type 2 diabetic patients, the incidence and severity of calcified plaques (coronary and/or carotid) was linked to multiple chromosomal sites. Using a subset of these families enriched for diabetic patients, the strongest evidence for linkage was with genetic markers on chromosome 3p. This linkage was not independent from traits of the metabolic syndrome (increased blood glucose, body weight, and dyslipidemia). Thus, genome scanning is identifying chromosomes harboring genes predisposing to atherosclerosis and diabetes. These studies support the concept that common genetic factors may predispose to both type 2 diabetes and atherosclerosis.

Genetic linkage studies in mice are providing a more rapid analysis of phenotype to genotype relationships as well as identifying traits that are colinked. Several studies have addressed whether distinct patterns of glucose versus lipid metabolism contribute to atherosclerosis. In a cross between PERA and C57BL/6 mice lacking the LDLR, quantitative trait loci for plasma lipids, body weight, insulin, and aortic root atherosclerosis were identified. The chromosome 4 quantitative trait loci contributed to variations in lipids and body weight with significant genotype effects on extent of atherosclerosis, suggesting that a common genetic mediator exists for these combined traits.

In contrast to the work above, there are 2 genome-scanning studies in mice in which traits for plasma glucose concentration and atherosclerosis lesion size are not correlated. A quantitative trait locus analysis was conducted in an F2 cross
between C57BL/6J and C57BLKS/J mice both carrying the deficiency of leptin receptor (db/db) to test the relationship between atherosclerosis and type 2–like diabetes. Separate genetic factors were identified for atherosclerosis (chromosome 12) and elevated glucose levels (chromosomes 8 and 17), supporting the idea that distinct genetic factors control each of these traits. The separation of glucose and atherosclerosis was also seen in a study of 2 congenic strains of C57BL/6 carrying loci derived from CAST/Ei (CAST). Two regions of chromosome 2 alleles from CAST mice were introgressed into the C57BL/6 background and subsequently bred to LDLR −/− mice to create hyperlipidemic backgrounds for the CAST alleles. Mice fed chow and Western diets were studied for measures of glucose and lipid homeostasis and atherosclerosis. The MOB5 congenic was insulin resistant and showed atherosclerosis comparable to LDLR −/− mice. However, plasma glucose levels were lowest for this strain, illustrating that elevated plasma glucose concentrations are not sufficient for promoting atherosclerosis in fat-fed mice with plasma cholesterol levels of 1000 mg/dL. In contrast, although the MOB6 congenic showed comparable glucose levels to the LDLR −/− strain, atherosclerosis was 2-fold reduced in this strain. Plasma lipids and lipoprotein profiles were nearly identical between MOB6 and MOB5 strains. This study illustrates that individual traits associated with the metabolic syndrome can be separated and that insulin resistance was more important for promoting atherosclerotic lesions than elevated plasma glucose levels.

Overall, diabetes or insulin resistance may amplify the risk associated with genetic susceptibility to atherosclerosis already present in individuals. It is still not clear whether diabetes susceptibility genes provide additional or unique genetic elements affecting atherosclerosis and cardiovascular disease. It remains a major challenge to dissociate such genes involved in lipid and glucose homeostasis.

**Do Glucose and Lipids Exert Independent Effects in Cultured Endothelial Cells or Macrophages?**

If elevated glucose indeed exerts biological effects that are distinct from those of elevated lipids, then one would expect to see different biological responses, or at least intracellular signaling events, to elevated glucose and lipids in vascular cells involved in atherosclerosis, such as endothelial cells and macrophages. This, however, is generally not the case. In many instances, the effects of glucose can be mimicked by long-chain fatty acids common in circulation (such as palmitate, oleate, and linoleate), or by oxidized phospholipids, which are present in modified LDL and are believed to play an important role in atherosclerosis.

In macrovascular endothelial cells, elevated levels of glucose have been shown to induce VCAM-1 expression, potentially explaining the effects of diabetes on macrophage accumulation in lesions of atherosclerosis. However, a stimulatory effect of glucose is not always seen, and experiments by other groups have not found increased VCAM-1 expression in human endothelial cells exposed to elevated glucose. These discrepancies may be attributable to differences in sources of cells, incubation time, and concentration of glucose; differences in posttranslational modification of proteins and lipids by glucose; presence or absence of insulin; or other factors related to the experimental design. Interestingly, serum samples from patients with diabetes did induce increased VCAM-1 expression under conditions in which glucose alone had no effect, suggesting that hyperglycemia alone might not explain the effects of diabetes on VCAM-1 expression. These results are consistent with in vivo studies, in which hyperglycemic mice do not develop early lesions unless plasma lipid levels are above a certain threshold. Age interaction with RAGE has also been shown to induce VCAM-1 in human endothelial cells, and injection of AGEs can induce VCAM-1 expression in rabbits in vivo. However, other studies have not observed VCAM-1 expression in AGE-stimulated endothelial cells, potentially because of the presence of different RAGE splice variants. Similarly, palmitate alone has been shown to induce VCAM-1 expression in macrovascular endothelial cells by some but not by others. High glucose has also been shown to increase monocyte adhesion to endothelial cells though a mechanism that involves increased interleukin (IL)-8 production. This effect of glucose is mimicked by minimally modified LDL or by specific oxidized phospholipids.

In human macrophages, elevated glucose has been shown to increase expression of the scavenger receptor and fatty acid transporter CD36, an effect that is mimicked by oleate and linoleate. However, a similar effect of glucose on CD36 has not been found in peritoneal macrophages from mice. It is possible that the different responsiveness of human and mouse macrophages to glucose could be attributable to the lower expression of aldose reductase in mouse macrophages, because increasing expression of aldose reductase in mouse macrophages results in increased CD36 expression. Other receptors involved in the uptake of modified LDL have also been demonstrated to be induced by glucose. In fact, glucose alone appears able to promote cholesterol accumulation in a macrophage cell line. Accordingly, monocytes from people with diabetes express elevated levels of CD36 and increased uptake in vitro of modified LDL. In addition, elevated glucose stimulates cellular lipid loading by reducing levels of ABCG1, which is involved in cholesterol efflux from macrophages. Oleate and linoleate result in a similar reduction of ABCG1 levels in macrophages.

Both type 1 and type 2 diabetes are associated with augmented inflammation, as indicated by increased secretion of proinflammatory cytokines from monocytes from these patients. Fatty acids and glucose can stimulate secretion of cytokines from macrophages. Elevated glucose levels induce secretion of IL-12 in mouse peritoneal macrophages, as well as elevate mRNA levels for IL-1β, IL-6, IL-12, and tumor necrosis factor-α. Saturated fatty acids, such as palmitate, induce tumor necrosis factor-α in a macrophage cell line. Together, these results suggest that the effects of elevated glucose levels in isolated endothelial cells and macrophages are often mimicked by lipids.
Elevated Glucose and Lipids Can Induce Similar Intracellular Signals in Endothelial Cells

One intracellular mechanism whereby glucose can mediate biological effects has been identified by Brownlee and colleagues. This group has shown that many of the effects of elevated glucose on bovine aortic endothelial cells are attributable to increased mitochondrial production of superoxide and subsequent inhibition of the glycolytic enzyme glyceraldehyde-3 phosphate dehydrogenase (GAPDH). Inhibitors of GAPDH activity by elevated glucose leads to accumulation of upstream glycolytic intermediates, such as glyceraldehyde-3-phosphate, which in turn can result in increased activity of the aldose reductase-dependent sorbitol pathway, increased DAG formation and protein kinase C (PKC) activation, increased flux through the hexosamine pathway, and increased levels of intracellular advanced glycation endproducts (AGEs). These "glucose-like" effects of fatty acids are shown schematically in Figure 2A. It is not yet known whether this phenomenon can be generalized to different cell types or species. In addition to fatty acids, minimally oxidized LDL and oxidized phospholipids also appear to mediate their effects in endothelial cells through increased superoxide generation.

Many of the Signaling Pathways Induced by Glucose Are Mediated by Bioactive Lipids

Interestingly, many of the intracellular signaling pathways shown to be induced by elevated glucose are mediated by bioactive lipids. This provides a plethora of interactions between glucose and lipids. We discuss 3 of these lipid mediators of glucose action: DAG, sphingosine 1-phosphate, and arachidonic acid metabolites, as summarized in Figure 2B.

It is well established that elevated glucose causes increased DAG synthesis and mass in tissues and in isolated cells. In the aorta of diabetic dogs, increased DAG levels are sustained for several years. Fatty acyl–coenzyme A (acyl-CoA) synthesis is required for DAG synthesis. Accordingly, it has been shown that chronic elevation of glucose increases acyl-CoA levels in rat skeletal muscle. How does glucose increase fatty acyl-CoA and DAG mass? King and colleagues have shown that the increased DAG mass occurs partly by de novo synthesis from glucose, probably via glyceraldehyde 3-phosphate and phosphatidic acid. The increased DAG is likely to explain the ability of glucose to activate DAG-sensitive protein kinase C isoforms, as well as downstream effects, such as decreased nitric oxide generation. One mechanism whereby glucose may increase DAG levels has been identified by Brownlee, as discussed above. In addition, Ruderman and colleagues have proposed a malonyl-CoA/long-chain acyl-CoA model that operates in several different cell types, including endothelial cells. Elevated glucose leads to increased levels of malonyl-CoA, which inhibits fatty acid β-oxidation by inhibiting mitochondrial fatty acyl-CoA uptake, thereby channeling increased levels of acyl-CoAs into DAG and triacylglycerol. Together, these studies indicate that elevated glucose causes increased DAG synthesis, probably by several different mechanisms that all require fatty acyl-CoA.

It has recently been shown that elevated levels of glucose can activate the sphingosine kinase pathway in aortic endothelial cells and that diabetes leads to a similar activation in the rat aorta. The glucose-induced increase in sphingosine kinase activity was shown to be attributable to selective activation of the sphingosine kinase 1 isoform. By using a dominant-negative mutant of sphingosine kinase 1, Wang and colleagues demonstrated that glucose-induced adhesion of monocytic cells was abolished in the absence of a functional sphingosine kinase 1. Activation of sphingosine kinase results in generation of sphingosine-1-phosphate (S1P). Interestingly, S1P was recently shown to inhibit monocyte adherence to aortas from diabetic NOD mice through activation of the S1P1 receptor. The overall biological effect of S1P is likely to depend on S1P receptor subtypes expressed under different conditions.
conditions. Thus, in addition to DAG, sphingolipids appear to mediate some of the effects of elevated glucose, at least in endothelial cells.

Elevated glucose levels have also been shown to regulate several arachidonic acid metabolites. In the context of elevated glucose, arachidonic acid metabolism by cyclooxygenases to prostanooids, and by lipoxygenases to hydroxyeicosatetraenoic acids (HETEs), are most relevant. Elevated glucose levels induce cyclooxygenase-2 expression in human aortic endothelial cells, and this is associated with an increased release of thromboxane B$_2$ and prostaglandin E$_2$. It has recently been shown that a thromboxane A$_2$ receptor antagonist protects against endothelial dysfunction and diabetes-accelerated lesion initiation in apoE-deficient mice. Elevated glucose also induces expression of 12/15 lipoxygenase and increases levels of the 12/15 lipoxygenase product 12(S)-HETE in aortic endothelial cells. Interestingly, overexpression of 15-lipoxygenase in the endothelium of LDLR-deficient mice results in larger fatty streaks compared with LDLR+/− controls. Accordingly, 12(S)-HETE has been found to stimulate monocyte adhesion to cultured endothelial cells. Minimally oxidized LDL and oxidized phospholipids also appear to mediate their effects in endothelial cells through increased arachidonic acid release and subsequent stimulation of the lipoxygenase pathway. In this context, it is interesting that elevated glucose can cause oxidation of LDL and that reactive carbonyl species, AGEs, and advanced lipoxidation end products (ALEs) also might result in modification of lipids in lipoproteins.

Conclusions and Perspective

It is clear that type 1 diabetes, without marked changes in plasma lipid levels, can accelerate atherosclerosis and cardiovascular disease in humans and experimental animal models. Elevated glucose might play an important role in this effect, but glucose is likely to act in concert with basal lipid levels. Thus, hyperglycemia alone appears insufficient to accelerate atherosclerosis, at least in various animal models. Similarly, when plasma lipid levels are extremely high, the effect of hyperglycemia appears to be overpowered, perhaps because glucose and lipids act, at least in part, by the same mechanisms to accelerate atherosclerosis. We propose that there is a significant amount of cross-talk between signaling events induced by glucose and lipids.

In the past 10 years, much has been learned regarding how diabetes accelerates lesion initiation, but little is still known about how diabetes affects more advanced lesions. Given the fact that different phases of lesion progression are regulated by different mechanisms, it is unlikely that diabetes accelerates cardiovascular events merely by inducing an accelerated formation of lesions of atherosclerosis. Studies on advanced lesions will be necessary to further our knowledge on the cellular and molecular mechanisms whereby diabetes leads to cardiovascular events.

Note Added In Proof

A recent study by Shi et al (J Clin Invest. 2006;116:3015–3025) shows that fatty acids stimulate cytokine production primarily through a Toll-like receptor 4 (TLR4)-dependent mechanism in macrophages. It will be important to evaluate the role of this TLR4 pathway versus intracellular effects of fatty acids in vascular cells.

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Disclosures

None.

References

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68. MacDougall ED, Kramer F, Polinsky P, Barnhart S, Askari B, Johansson L162V.


76. MacDougall ED, Kramer F, Polinsky P, Barnhart S, Askari B, Johansson L162V.


129. Laybutt DR, Schmitz-Pfeiffer C, Saha AK, Ruderman NB, Biden TJ, Kraegen EW. Muscle lipid accumulation and protein kinase C activation


Do Glucose and Lipids Exert Independent Effects on Atherosclerotic Lesion Initiation or Progression to Advanced Plaques?

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