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Diabetic vascular disease and the potential role of macrophage glucose metabolism

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Abstract

Cardiovascular complications remain the leading cause of mortality in adult human subjects with diabetes. Hyperglycemia has long been hypothesized to explain some of the effects of diabetes on cardiovascular complications caused by atherosclerosis, but a clear causative role for hyperglycemia has not been established. Recent studies in animal models indicate that glucose may play a role in diabetes-accelerated atherosclerosis by promoting pro-inflammatory responses in myeloid cells, which are key cell types in atherosclerosis. For example, monocytes and macrophages often take on a more pro-inflammatory phenotype in the setting of diabetes. Moreover, *in-vitro* studies demonstrate a connection between pro-inflammatory molecules and glucose metabolism in macrophages and dendritic cells. This review concerns the role of glucose metabolism in inflammatory macrophages, and their potential role in diabetic vascular disease. Further *in-vivo* studies, focusing on myeloid-specific effects of glucose metabolism as it relates to atherosclerosis, are needed to increase our understanding of the relationship between diabetes, myeloid cells, and cardiovascular disease.

Keywords

Atherosclerosis; diabetes; glycolysis; hyperglycemia; inflammation; macrophage; mouse

Clinical studies and animal studies suggest a role for hyperglycemia in diabetic vascular disease, but only under certain conditions

Many clinical studies demonstrate a correlation between suboptimal glycemic control and cardiovascular events in patients with type 1 diabetes (1–3), although this relation is not consistently observed (4–6). One issue, which has recently been brought to light, is that levels of glycated hemoglobin A_{1c} (HbA_{1c}), the current standard for analysis of glycemic control, have been shown to be regulated by genetic components, some of which are unrelated to elevated glucose levels (7). Thus, levels of HbA_{1c} might not always be a good measure of the extent of glycemic control. Another issue is that cardiovascular disease develops over long periods of time, and clinical intervention studies are rarely conducted over the time-span of several years or decades. Interestingly therefore, long-term follow-up studies provide the most compelling evidence for a beneficial effect of improved glycemic control on cardiovascular end-points (2,8). Furthermore, it is difficult to achieve optimal glycemic control in clinical practice. One interesting long-term study is the follow-up of the

Diabetes Control and Complications Trial cohort. Strikingly, in this long-term follow-up, the effect of tight glycemic control early in the study led to significantly beneficial cardiovascular outcomes many years later in subjects with type 1 diabetes (2).

Patients with type 2 diabetes often show increases in a number of cardiovascular risk factors, such as dyslipidemia, hypertension, and insulin resistance. Clinical intervention to improve either dyslipidemia or hypertension demonstrates the reduction of cardiovascular events, whereas the effect of glycemic control is controversial. Thus, hyperglycemia may play a less important role in patients with type 2 diabetes, as compared to patients with type 1 diabetes. Indeed, intensive blood glucose lowering in patients with type 2 diabetes has been shown to be either beneficial, to be without effect, or even to be detrimental to the development of cardiovascular events (9–12). Although several other risk factors are likely to play a more important role than glucose in patients with established type 2 diabetes, epidemiological studies strongly indicate an association between blood glucose levels and the risk of cardiovascular disease (13), and it cannot be ruled out that hyperglycemia plays a role, especially in patients with relatively normal lipids and few other risk factors.

Can we extrapolate information from animal studies to get a better understanding of the role of hyperglycemia in human cardiovascular disease? In mice, hyperglycemia is associated mainly with an increased formation of early macrophage-rich lesions of atherosclerosis (14–16). It is therefore possible that the principal effect of hyperglycemia is to accelerate the first stages of lesion development (lesion initiation) and that this effect, perhaps together with other risk factors, could lead to the occurrence of cardiovascular events earlier in life in subjects with diabetes as compared with subjects without diabetes. The insight provided by long-term follow-up studies (2,8) may be consistent with these findings in mice, although several other explanations are possible. When tight glycemic control was started early in the course of type 1 diabetes (2), likely before the development of advanced lesions, the group receiving intense treatment to improve glucose control might have developed lesions at a slower rate, which resulted in fewer cardiovascular events after several years, even though at this time, blood glucose levels were similar between the groups. Another hypothesis for how elevated blood glucose levels might exert detrimental effects years later, even when glucose levels have been normalized, is that elevated glucose might induce long-term epigenetic changes in tissues susceptible to development of complications of diabetes (17), or through increased formation of advanced glycation end-products.

In diabetic animals that are also severely dyslipidemic, a stimulatory effect of diabetes independent of that of dyslipidemia is often absent (18–23). These animal models might therefore mimic some of the aspects of type 2 diabetes, in which dyslipidemia masks and overrides the stimulatory effects of hyperglycemia.

Thus, mouse models suggest that hyperglycemia may play a role in initiation of macrophage-rich atherosclerotic lesions, potentially leading to more advanced lesions later in life. Recent studies on new mouse models have begun to provide interesting evidence that glucose indeed does affect vascular cells *in vivo*.

How can direct effects of elevated glucose on vascular cells be evaluated in animal models?

How can the effect of glucose in different cell types within the atherosclerotic lesion be tested *in vivo*? One interesting approach was recently taken to address whether increased glucose uptake specifically in vascular smooth muscle cells would alter these cells' properties *in vivo* (24). In this study, the glucose transporter 1 (GLUT1; also known as SLC2A1) was over-expressed in smooth muscle cells in a transgenic mouse model. When

mice with GLUT1 overexpressing smooth muscle cells were subjected to femoral artery injury, an increased accumulation of neutrophils was observed in the injured vessel (24). Thus, increased glucose uptake in smooth muscle cells appears to enhance their pro-inflammatory potential. Although the vascular injury model used in this study should not be equated with atherosclerosis, these findings provide a first important view into the cell type-specific effects of glucose *in vivo*. It would be informative to evaluate atherosclerosis in mice with increased glucose uptake specifically in smooth muscle cells and other vascular cells involved in atherosclerosis.

Previous studies have used mouse models to investigate the role of advanced glycation end-products (AGEs) and the receptor of AGEs (RAGE) in atherosclerosis. These models did not provide cell type-specific data but demonstrated clearly that loss of RAGE prevents atherosclerosis in diabetic dyslipidemic mice (21,25). Subsequent studies demonstrated that RAGE deficiency also protects against atherosclerosis in non-diabetic mice and that RAGE binds several types of ligands (26). Bone-marrow transplant studies suggested that the protective effects of RAGE deficiency in non-diabetic apoE^{-/-} mice is due partially to RAGE expression in bone-marrow-derived cells (27). Thus, RAGE activation does not occur only in diabetes, and RAGE does not specifically mediate effects of hyperglycemia but is likely to have a more wide-spanning importance in disease states associated with increased inflammation.

Another approach is to study enzymes in glucose metabolism believed to mediate the effects of glucose. An excellent example of this approach is the human aldose reductase (AR) transgenic mouse (15). This mouse expresses levels of aldose reductase similar to those observed in humans, and exhibits increased atherosclerosis specifically in the setting of diabetes (15). Thus, it is possible that hyperglycemia promotes atherosclerosis, at least in part, through increased flux through the polyol/sorbitol pathway (see Figure 1). It is not yet known what cell type might be primarily responsible for this effect.

These new mouse models, and other similar models, will provide much-needed insight into the potential role of glucose in different vascular cells in atherosclerosis *in vivo*.

High glycolysis in macrophages in atherosclerotic lesions

There is evidence that acutely elevated glucose levels promote leukocyte–endothelial interactions *in vivo* (28), particularly in the presence of inflammatory mediators (29). This effect of glucose could potentially provide an important first step in glucose-induced lesion formation. Myeloid cells—monocytes, macrophages, and dendritic cells—are major players in the immune system and rapidly accumulate at sites of inflammation, such as lesions of atherosclerosis (30,31). These sites are often characterized by poor vascularization and hypoxic conditions. Thus, in addition to lesions of atherosclerosis, high numbers of macrophages have been reported in joints affected by rheumatoid arthritis (32) and in hypoxic areas of dermal wounds (33). Macrophages must be able to move into a hypoxic environment during inflammation and to carry out their varied functions under such conditions, which requires substantial amounts of ATP.

Under normoxic conditions, most cells produce ATP from glucose via generation of pyruvate through glycolysis and subsequently use pyruvate to fuel the citric acid (TCA) cycle and oxidative phosphorylation. This is an efficient method of generating energy, since it theoretically results in 38 ATP molecules/glucose molecule used. However, under hypoxic conditions the cell's energy demand is met by glycolysis and conversion of pyruvate to lactate, rather than channeling into the citric acid cycle. This process produces only two ATP molecules from one glucose molecule, and is therefore an inefficient process, as compared to oxidative phosphorylation. Although macrophages and dendritic cells depend

heavily on glycolysis for ATP production even under normal oxygen tension, these cells adapt to low oxygen tension by increasingly utilizing the anaerobic glycolytic pathway for ATP production (34,35). In isolated mouse peritoneal macrophages, glucose contributes to ATP production to a greater extent than does oleate (36), and almost all of the utilized glucose is converted into lactate through glycolysis, whereas only ~3% of glucose is oxidized under oxygenated conditions (37). Furthermore, hypoxia stimulates glycolysis and prolongs cell survival in mouse bone-marrow-derived macrophages and human monocyte-derived macrophages (38). These data suggest that glucose utilization and the glycolytic pathway might influence the activities of macrophages at sites of inflammation, such as atherosclerotic lesions (39,40).

Before a necrotic core has started to form, a large atherosclerotic lesion typically has a macrophage-rich core that has a high metabolic rate, and these macrophages are often restricted to anaerobic metabolism (41). In fact, a high degree of anaerobic glycolysis was observed in rabbit lesions, in which high lactate production and high glucose consumption were seen in the core of advanced lesions (42). The high glucose consumption is predicted to result in a reduction of glucose levels in the core of poorly vascularized large lesions, but absolute levels of glucose in macrophage-rich lesions in non-diabetic and diabetic settings are difficult to assess. Emerging imaging technology, using fluorine-18-labeled-2-deoxy-D-glucose positron emission tomography (FDG-PET) has also shed light onto the metabolic features of lesion macrophages. A number of studies have reported an increased uptake of FDG in atherosclerotic lesions (43). There is a general agreement from these studies that the arterial FDG signal is proportional to the macrophage content of the lesion, although a few negative reports exist, presumably due to the non-specific nature of FDG-PET imaging (43). High FDG uptake in atherosclerotic lesions has also been demonstrated in human subjects (44). Generally, the degree of lesion FDG uptake increases with the number of cardiovascular risk factors, including diabetes (43,45).

Cellular glucose uptake is mediated by a family of facilitated diffusion glucose transporters, the GLUT or SLC2A family, and of these GLUT1 through 4 have specific and well established roles in glucose homeostasis (46). Of these four GLUT isoforms, monocytes/macrophages from different species have been shown to express GLUT1 and GLUT3 (47–49), with significant increases in GLUT1 expression during differentiation (47,48). GLUT4, the main insulin-stimulated glucose transporter (46), is abundantly expressed in skeletal muscle and adipose tissue and is not observed in human monocyte/macrophages (47). Glucose uptake in macrophages is therefore not critically dependent on insulin.

Once glucose has entered the cell, most of it is believed to be channeled into glycolysis. Glycolysis is regulated by three major enzymes: hexokinase, phosphofructokinase 1 (PFK1), and pyruvate kinase, which mediate irreversible steps in the pathway (Figure 1). Hexokinase converts glucose to glucose-6-phosphate. Glucose-6-phosphate is then converted to fructose-6-phosphate by glucose-6-phosphate isomerase and then further to glucose-1,6-bisphosphate by PFK1. PFK1 is a rate-limiting enzyme in glycolysis and is regulated by several factors. Increased levels of ATP and citrate inhibit PFK1 activity, whereas ADP, AMP, and fructose-2,6-bisphosphate act as allosteric activators of PFK1. Importantly, fructose-2,6-bisphosphate, a strong activator of PFK1, is generated from fructose-6-phosphate by PFK2. PFK2 contains both kinase and phosphatase domains. Under low glucose conditions, the phosphatase is active, thus lowering fructose-2,6-bisphosphate levels, which in turn slows glycolysis. When glucose levels are high, the kinase domain is active, producing more fructose-2, 6-bisphosphate, which stimulates glycolysis. There are four subtypes of PFK2 reported in higher mammals, and each subtype has its own net activities and ratios of kinase/bisphosphatase activity (50,51). Therefore, the net activity of

PFK2 is believed to influence largely the activity of PFK1 and, consequently, the activity of glycolysis.

Several of the glycolytic proteins are highly expressed or activated in macrophages. For example, the enzymatic activity of hexokinase in mouse peritoneal macrophages is reported to be high (52). Hypoxia-inducible factor-1 (HIF-1) is a key regulator of the expression of genes necessary for adaptation to hypoxic conditions. Most of the glycolytic pathway-related genes are induced by HIF-1, including GLUT1, hexokinase, PFK, and phosphoglycerate kinase 1 (53,54). Loss of HIF-1 α in macrophages by targeted disruption of the gene resulted in the reduction of glycolytic activity and ATP production to 20% of that observed within wild-type cells (55). The activity of HIF-1 is regulated by factor inhibiting HIF-1 (FIH-1) and prolylhydroxylases. FIH-1 and prolylhydroxylases are oxygen sensors, and in most cells HIF activity is strongly suppressed by the action of these proteins under normoxic conditions (56–59). However, macrophages and dendritic cells primarily use glycolysis for ATP production even under normoxic conditions. Recently it was demonstrated that in bone-marrow-derived macrophages, the adapter protein Mint3/APBA3 binds to FIH-1 and suppresses the activity of this protein, consequently enhancing the activity of HIF-1 (60). Furthermore, membrane type 1 matrix metalloproteinase, a potent invasion-promoting protease expressed in macrophages, was shown to bind FIH-1 and lead to the inhibition of this protein even under normoxic conditions through a Mint3/APBA3-dependent pathway (61).

Together, these studies demonstrate that macrophages utilize primarily glycolysis for energy production under both hypoxic and normoxic conditions.

There is a strong connection between glucose metabolism and a pro-inflammatory phenotype in macrophages

Recent research has revealed that pro-inflammatory activation of macrophages regulates their energy metabolism as well as inflammatory phenotype and that fuel utilization and inflammatory phenotype are closely related in these cells. Activation of macrophages has been classified into different states or populations *in vitro*, depending on the stimulus (62,63). The classical (M1) activation results in a highly pro-inflammatory macrophage phenotype, is a feature of microbicidal activity and pro-inflammatory cytokine production, and is mediated by like Toll-like receptor (TLR)-4 ligands and interferon- γ (IFN- γ). The alternative (M2) activation is a feature of a less inflammatory reaction, tissue repair and humoral immunity, and is thought to be mediated by IL-4 and/or IL-13. It was recently demonstrated that there is a clear difference in the flux of glucose metabolism between the classical activation and alternative activation (64,65). Interestingly, inflammatory classically activated macrophages are more dependent on glucose as a substrate, whereas the less inflammatory alternatively activated macrophage population is more dependent on fatty acid metabolism (64). Although the glycolytic pathway gives a lower ATP yield than the TCA cycle/oxidative phosphorylation, glycolysis is believed to be able rapidly to provide ATP to macrophages, which need to meet the increased energy demand associated with defense against pathogens/infection (64). Furthermore, pharmacological inhibitor studies have revealed that mitochondrial respiration is required for the alternatively activated macrophage phenotype, but not for the classically activated macrophage phenotype (64). A similar increase in aerobic glycolysis has recently been observed in dendritic cells stimulated with TLR ligands (35).

How do cytokines and pathogens promote glycolysis? It is possible that part of the effect is due to an increased expression of GLUT1 (66), which would be likely to increase glucose uptake and subsequent glycolysis. Furthermore, an inducible form of PFK2 (PFKFB3) has

caught attention because this enzyme is induced by hypoxia, inflammatory cytokines, and pathogens alike (67). Classical activation of macrophages shifts the isoform expression of PFK2 from the liver type PFK2, which has low net activity, to the more active ubiquitous PFK2 (PFKFB3), which keeps fructose-2,6-bisphosphate concentration at a high level due to its lower bisphosphatase activity, as compared to the liver type PFK2 (68,69). Consequently, this shift potentiates glycolytic ATP production to be utilized for pro-inflammatory responses in the classically activated macrophages. The PFKFB3 promoter contains putative HIF-binding sites, as well as an NF- κ B site (67). The effect of cytokines on induction of PFKFB3 does not appear to be due to HIF activation (65) and is likely to be mediated by the NF- κ B pathway. The increased glucose utilization in macrophages exposed to pro-inflammatory mediators could therefore be due in part to an increased PFKFB3 expression. The TLR4 ligand lipopolysaccharide (LPS) has also been shown to increase expression of glucose-6-phosphate dehydrogenase (G6PD) in macrophages (70). G6PD therefore provides an additional enzyme induced by pro-inflammatory mediators, and its induction might increase glucose flux through the pentose phosphate pathway (Figure 1).

The findings above demonstrate that pro-inflammatory molecules stimulate glucose metabolism/glycolysis in macrophages. Conversely, increased glucose uptake in macrophages can promote or contribute to a pro-inflammatory phenotype, suggesting that a pro-inflammatory environment and increased glucose levels might enhance each other in a vicious or self-perpetuating cycle. For example, recent studies suggest that down-regulation of PFKFB3 results in reduced expression of markers for the classically activated macrophage phenotype (65), indicating that PFKFB3 activity promotes a pro-inflammatory response. However, PFKFB3^{-/-} mice exhibit increased insulin resistance, and macrophages isolated from the adipose tissue of these mice showed an increased production of cytokines (71), possibly mediated by the pro-inflammatory *in-vivo* environment. The role of PFKFB3 induction in mediating increased inflammatory activation of macrophages therefore needs further study.

Consistent with the findings that glucose is poorly utilized in mitochondrial respiration in pro-inflammatory macrophages, pharmacological inhibition of mitochondrial respiration does not alter the macrophage's pro-inflammatory phenotype (64), suggesting that glucose metabolism through mitochondrial respiration does not mediate the pro-inflammatory effects of glucose (Figure 1).

In addition to the glycolytic pathway, increased glucose uptake might result in regulation of the macrophage's inflammatory phenotype, e.g. through the pentose phosphate pathway, the polyol/sorbitol pathway, or the hexosamine pathway (Figure 1). Increased expression of G6PD increases glucose flux through the pentose phosphate pathway in macrophages, which associates with an enhanced inflammatory response in obesity (72). LPS stimulation leads to G6PD up-regulation in macrophages, and over-expression of G6PD increases the expression of inflammatory markers, such as MCP-1, IL-6, and iNOS (72). Mononuclear cells from G6PD-deficient human subjects produce reduced levels of cytokines (73), further suggesting that this enzyme or downstream processes have a pro-inflammatory effect. G6PD-deficient mice have been shown to have reduced atherosclerosis, although the role of macrophages in this response is unknown (74).

Likewise, the polyol/sorbitol pathway can mediate increased production of pro-inflammatory mediators in macrophages (75). Aldose reductase, a key enzyme in this pathway, has been shown to promote LPS-induced pro-inflammatory responses in mouse macrophages. Inhibition or ablation of the activity of aldose reductase resulted in marked suppression of LPS-induced production of inflammatory cytokines (75), whereas over-expression of aldose reductase resulted in increased expression of pro-inflammatory

mediators in macrophages (15). Furthermore, aldose reductase expression and activity are increased by foam cell formation induced by oxidized LDL in human monocyte-derived macrophages, and these effects are potentiated by high glucose conditions (76). Importantly, both the polyol/sorbitol pathway and the pentose phosphate pathway feed into glycolysis, and these pathways are therefore closely related to glycolysis (Figure 1).

There is a little information regarding the effect of the hexosamine biosynthesis pathway on macrophage inflammation, but one study concluded that this pathway does not significantly alter LPS-induced iNOS expression, a marker of classically activated macrophages (77). The contribution of the hexosamine pathway in pro-inflammatory responses requires further study.

Together, these studies suggest that a glycolytic intermediate(s), perhaps with contribution from the polyol/sorbitol pathway and the pentose phosphate pathway, is the most likely mediator of the pro-inflammatory effects of glucose in macrophages (Figure 1). The exact mechanisms whereby this might occur need further study.

Hyperglycemia may activate a pro-inflammatory program by mimicking the increased glycolysis induced by cytokines and pathogens in macrophages

Several human and animal studies show that monocytes/macrophages take on a pro-inflammatory phenotype in the setting of type 1 diabetes (78–81), and this effect might be mediated, at least in part, by increased glucose levels (80). It is tempting to speculate that diabetes, through hyperglycemia and subsequently increased macrophage glucose uptake, mimics the stimulatory effects of cytokines and pathogens on glycolysis in macrophages, thereby promoting a pro-inflammatory response in these cells, which would likely promote atherosclerosis. In addition, diabetes is often associated with a pro-inflammatory environment, which could in turn further promote macrophage glucose metabolism. Studies on new mouse models, for example models in which GLUT1 or glycolytic enzymes are modulated specifically in macrophages, and ultimately human studies, will be required to gain further understanding of the role of glucose in vascular cells and atherosclerosis *in vivo*.

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Abbreviations

AGE	advanced glycation end-product
AR	aldose reductase
FDG-PET	fluorine-18-labeled-2-deoxy-D-glucose positron emission tomography
FIH-1	factor inhibiting HIF-1
G6PD	glucose-6-phosphate dehydrogenase
GLUT1 (SLC2A1)	glucose transporter 1
HbA_{1c}	glycated hemoglobin A _{1c}
HIF-1	hypoxia-inducible factor-1
IFN	interferon

IL	interleukin
LPS	lipopolysaccharide
PFK	phosphofructokinase
RAGE	receptor for AGE
TLR	Toll-like receptor

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Key messages

- Increased glucose metabolism in macrophages might contribute to the increased atherosclerosis associated with diabetes.

Figure 1.

Schematic representation of the potential links between diabetes, glucose metabolites, and pro-inflammatory effects in macrophages. Macrophages rely heavily on glycolysis as an energy source, both under aerobic and anaerobic conditions. Diabetes is likely to increase glucose uptake in macrophages. Cytokines and pathogens also stimulate glycolysis by increasing expression of GLUT1, G6PD, and PFKFB3. Diabetes could therefore potentially stimulate glucose flux in macrophages through two mechanisms, by a direct effect through hyperglycemia, and by promoting a pro-inflammatory environment, which in turn further enhances glucose metabolism in a self-perpetuating cycle. Recent research shows that the pro-inflammatory phenotype of macrophages associated with diabetes might be due to increased glucose flux through the polyol/sorbitol pathway, the pentose phosphate pathway, and/or glycolysis, whereas mitochondrial respiration does not appear to affect the pro-inflammatory phenotype. The polyol/sorbitol pathway and pentose phosphate pathway both feed into glycolysis (as indicated by the gray box) and therefore could contribute to the increased flux through glycolysis.