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Does elevated glucose promote atherosclerosis? Pros and Cons

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"Dripping water hollows out stone, not through force but through persistence."

— *Ovid*

The quote above, by the Roman poet Publius Ovidius Naso (43 BC – 17 AD), has been an inspiration to me since childhood. It reminds me that accumulating pieces of evidence to tackle difficult questions will in time make an impact. Of course, some discoveries happen suddenly and unexpectedly while others take lifetimes. A question my laboratory has long examined is whether elevated glucose promotes atherosclerosis by acting directly on vascular cells in lesions of atherosclerosis in the setting of diabetes.[1] This problem appears to belong to the category Ovid might have had in mind when he coined the quote above. The reason we keep tackling this question from different angles and by different methods is the increased risk of cardiovascular complications associated with type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). There is still no consensus on how diabetes promotes atherosclerosis and resulting cardiovascular events, and whether glucose has a direct pro-atherosclerotic effect. Considerable time and resources have been devoted to studies of effects of glucose in isolated cultured vascular cells. In my opinion, for reasons described below, we now need to tackle the role of elevated glucose, hyperglycemia and glucose fluctuations by using animal models and studies in humans.

Studies in isolated vascular cells and animal models have begun to reveal that inhibition of glucose uptake and utilization in cells involved in atherosclerosis can prevent pro-atherosclerotic events in the absence of diabetes. This is not surprising since cellular activation often is associated with metabolic reprogramming. Thus, reducing expression of the glucose transporter GLUT1 in macrophages or hematopoietic cells limits inflammatory activation of macrophages, expansion of hematopoietic cells, and atherosclerosis in mice.[2, 3] Other studies demonstrate that disruption of glycolytic flux is detrimental to cells, and leads to inflammasome activation and pyroptosis in macrophages.[4]

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The question of whether *increased* levels of blood glucose provide a pro-atherosclerotic effect is a quite different issue. In this *Viewpoint* article, pros and cons regarding a direct vascular pro-atherosclerotic effect of elevated glucose are discussed and summarized in Table 1 with the hope of inspiring the reader to think about this topic with an open mind, to make his/her own conclusions, and to devise new approaches to address this issue *in vivo*.

Evidence from human studies

There is no question that improved glycemic control results in reduced retinopathy, nephropathy and neuropathy in subjects with T1DM or T2DM, but what are the pros and cons for a pro-atherosclerotic effect of elevated glucose in humans? The strongest evidence supporting a pro-atherosclerotic effect comes from the DCCT/EDIC study, in which young subjects with T1DM were divided into two groups, one treated with conventional insulin therapy and one treated with a more intensive insulin regimen, resulting in improved blood glucose control (Table 1).[5] After the DCCT treatment arm of the study, most of the subjects were followed in the EDIC observational arm. During follow-up, blood glucose control returned to similar levels in the two groups, but cardiovascular events were significantly reduced years later in the group that had received intense insulin therapy. Interestingly, recent studies have revealed that although many conventional cardiovascular disease risk factors apply in T1DM, hyperglycemia measured as glycated hemoglobin (HbA1c) is an important cardiovascular risk factor second only to age.[5] Of course, these studies do not necessarily prove causality, nor do they provide information on mechanism(s) whereby elevated glucose might promote atherosclerosis.

Conversely, many studies in subjects with T2DM suggest that cardiovascular events are not reduced by improved blood glucose control.[6] These studies suggest that if hyperglycemia is indeed directly responsible for exacerbating atherosclerosis in subjects with diabetes, its effects might be masked when other stronger risk factors are present, especially in subjects with T2DM. Other findings also line up on the con side of table 1. For example, patients with inactivating glucokinase mutations have mild fasting hyperglycemia from birth, resulting in an elevated HbA1c level. These patients do not demonstrate increased cardiovascular risk.[7] Furthermore, the EMPA-REG study on the effect of a sodium-glucose cotransporter 2 (SGLT2) inhibitor (empagliflozin) in subjects with T2DM has recently provided evidence that the reduced mortality resulting from the use of this SGLT2 inhibitor may not be due to reduced atherosclerotic events, but rather to altered hemodynamics, causing an impressive protection against heart failure,[8, 9] although it cannot be ruled out that empagliflozin acts in part by stabilizing atherosclerotic lesions. SGLT2 inhibitors act by inhibiting glucose reabsorption in the kidney with an increased glucose excretion and blood glucose-lowering effect as a result. We are now awaiting confirmation of these findings by other SGLT2 inhibitors.

Evidence from animal studies

Studies of animal models of diabetes often conclude that pro-atherosclerotic effects of the diabetic state are mediated by hyperglycemia or fluctuating glucose levels. There are a few studies that support this concept. Thus, transgenic LDL receptor-deficient ($Ldlr^{-/-}$) mice

expressing human levels of the enzyme aldose reductase (mice normally express lower levels of this enzyme, which catalyzes generation of sorbitol from glucose, than do humans) exhibit increased atherosclerosis when the mice are also diabetic.[10] These results support a role of glucose flux through the sorbitol pathway as a pro-atherosclerotic pathway, although it cannot be completely ruled out that aldose reductase acts on other aldehydes (Table 1).

Furthermore, recent studies have taken advantage of the SGLT2 inhibitors that have now been approved for blood glucose lowering in patients with T2DM. Since insulin promotes not only glucose uptake into insulin target tissues but also exerts important effects on e.g. lipids and gene expression, the SGLT2 inhibitor approach can be informative regarding effects of elevated glucose levels independent of changes in insulin. A recent study convincingly demonstrated that SGLT2 inhibitors lowered blood glucose levels in diabetic mice and concomitantly prevented the stimulatory effects of diabetes on myelopoiesis, inflammatory activation of lesional macrophages, and the inhibitory effects of diabetes on atherosclerotic lesion regression.[11] These effects of the SGLT2 inhibitors were likely mediated by glucose lowering. Alternatively, it is possible that the effects could have been mediated by indirect mechanisms, for example by hemodynamic changes,[8] rather than by prevention of direct effects of glucose on bone marrow cells or lesional cells.

While the studies discussed above support a role for hyperglycemia in promoting atherosclerosis associated with diabetes, a number of convincing studies have shown that hyperglycemia is not sufficient to promote atherosclerosis and does not always exert pro-atherogenic effects. Early studies performed in alloxan-diabetic rabbits demonstrated no atherogenic effect of hyperglycemia, but rather an inhibitory effect.[12] It was later postulated that this inhibitory effect of diabetes was due to generation of lipoprotein particles too large to enter the artery wall. Regardless of the mechanism, these results clearly show that hyperglycemia is not necessarily pro-atherogenic. A similar lack of hyperglycemia in promoting atherosclerosis have been shown in diabetic $Ldlr^{-/-}$ mice fed a high-fat diet[13] and in diabetic hypercholesterolemic minipigs.[14] Thus, hyperglycemia is not sufficient to promote atherosclerosis. This might be due to the fact that dyslipidemia is a much stronger driver of atherosclerosis than is diabetes *per se*, and that in hypercholesterolemic models, the effects of diabetes are masked, as has been demonstrated in diabetic hyperlipidemic mice.[1, 15] In addition, forcing myeloid cells to increase glucose uptake and glycolysis through overexpression of GLUT1 did not result in increased atherosclerosis or mimic the effect of diabetes on myeloid cells in $Ldlr^{-/-}$ mice.[3] It is possible that other cell types in lesions of atherosclerosis are more responsive to the effects of increased glucose.

Evidence from isolated cells relevant to atherosclerosis

A large number of studies on effects of elevated glucose levels in cultured cells relevant to atherosclerosis, such as endothelial cells, macrophages and arterial smooth muscle cells (SMCs), have been published and continue to be published. These studies are far too numerous to cite here. Suffice it to say that such studies have convincingly demonstrated that elevated glucose (often supplied at concentrations of 25 mmol/L or less), as compared to normal glucose (5–6 mmol/L), exerts effects that could be consistent with a pro-atherogenic effect. For instance, elevated glucose has been shown to increase expression of adhesion

molecules in endothelial cells through increased oxidative stress. These findings are consistent with the effect of diabetes on increased endothelial cell adhesion molecule expression and monocyte recruitment to the lesion.[11] Elevated glucose has also been shown to lead to increased cytokine and chemokine release from macrophages, mimicking the pro-inflammatory effects of diabetes. SMC proliferation and migration have been shown by a plethora of studies to be enhanced by elevated glucose. Such effects could potentially explain increased formation of fibrotic lesions in diabetes.

Whereas there is no reason to doubt these results, caution must be applied when interpreting cell culture studies because culture conditions are very different from *in vivo* conditions. A few issues we have considered are: First, cultured cells can exhibit changes in metabolism that do not reflect their metabolism *in vivo*. Cells in culture are often highly proliferative, which is often associated with metabolic reprogramming resulting in increased aerobic glycolysis. Second, to what extent are the cell cultures in question consuming glucose? It is not unusual for cells to consume a significant portion of glucose provided in the medium during the course of an experiment. As an example, human arterial SMCs consumed sufficient glucose to reduce glucose concentration from 5.6 mmol/L to 2.7 mmol/L in 24 h and to completely deplete the medium of glucose during a 48 h-period.[16] In order to perform these types of experiments, media should be changed frequently or glucose should be replenished by other methods. Third, relevant osmotic controls need to be carefully considered. L-glucose and mannitol are often used as osmotic controls. Whereas these types of controls are important, they are not physiological and could therefore exert unwanted effects. Forth, what other energy sources are present in the medium, and are levels of these substrates in the physiological range? Culture media often contain superphysiological concentrations of e.g. pyruvate and amino acids, which can be used by cells instead or in addition to glucose.[17] Fifth, there are sometimes significant differences between media batches, which could alter the biological responses of cells. For example, different levels of endotoxin could result in different extent of macrophage activation. Endotoxin could also be inadvertently introduced by spiking in glucose contaminated by endotoxin. Sixth, under conditions in which glucose has a significant biological effect, can increased glucose utilization or glucose flux be verified? Cells have mechanisms to protect themselves against excess glucose exposure. One of these mechanisms results in downregulation of the glucose transporter GLUT1 in some cell types exposed to high glucose levels.[18] Furthermore, how does increased glucose flux affect other substrates and metabolic pathways in the cell, including fatty acids and amino acids? Cells involved in atherosclerosis are, like many other cells in the body, metabolically flexible, meaning that they can and do readily shift the relative use of one energy source for another depending on availability and activation state of the cell. One well-known example of this phenomenon is the increased glycolysis observed during classical activation of macrophages by lipopolysaccharide (a component of the cell wall of Gram-negative bacteria) or other pathogens, which helps the macrophages fight bacterial infection.[19]

Together, cell culture studies on the effects of elevated glucose levels can be valuable when carefully monitored and performed in combination with *in vivo* animal studies or human studies.

Clinical implications and future directions

Despite many years of study it is still unclear whether elevated glucose can exert direct pro-atherogenic effects on cell types present in lesions of atherosclerosis *in vivo*. In this context, it should be emphasized that suboptimal blood glucose control is well-known to contribute to microvascular complications of diabetes (including eye complications and renal complications), and that adhering to optimal glucose-lowering therapies is critical for all subjects with diabetes and prediabetes. Optimal glycemic control will also improve other aspects of diabetes. Thus, in a sense, it does not really matter for the daily maintenance of diabetes if elevated glucose has adverse effects on lesional cells. It does matter, however, for our ability to develop new strategies for prevention and treatment of macrovascular/cardiovascular complications of diabetes.

It is possible that elevated glucose acts primarily on other tissues (liver, adipose tissue etc) and that effects on these tissues produce secondary effects on lesional cells that are more important than any direct effects of elevated glucose. It is also possible that elevated glucose acts primarily through extracellular mechanisms, e.g. by causing glycation and glycooxidation of proteins that can act as ligands for the receptor for advanced glycation end-products.

Cardiovascular disease associated with diabetes is a multifactorial disease, and diabetes is associated not only with hyperglycemia but with unphysiological glucose fluctuations, alterations in lipids, changes in hormones in addition to insulin, and often with a pro-inflammatory state. Dyslipidemia and other known cardiovascular risk factors are likely to have a greater impact that that of hyperglycemia in most patients.

Increased levels of inflammatory molecules in diabetes are likely to increase expression of the GLUT1 glucose transporter and the enzymes involved in glucose metabolism in lesional cells. Thus, elevated glucose uptake in these cells might primarily be a result of increased inflammation rather than a direct effect of hyperglycemia. Metabolomic studies of lesional cells, combined with epigenetic analysis, proteomic analysis, and RNA sequencing, are emerging as new ways to increase our knowledge in large animal and human subject populations. These techniques and approaches will lead to deeper understanding of the effects of diabetes on metabolism in lesional cells.

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Table 1

Does elevated glucose promote atherosclerosis? – Pros and cons

Human studies
Pros
• The DCCT/EDIC studies identified HbA1c as a strong risk factor for cardiovascular events in subjects with T1DM second only to age.
Cons
• Large studies have failed to demonstrate a clear beneficial effect of glucose-lowering therapies on cardiovascular disease in subjects with T2DM.
• A strong correlation between improved HbA1c and reduced risk of cardiovascular events does not necessarily imply causality or direct effects of glucose on the arterial wall.
• The SGLT2 inhibitor empagliflozin results in reduced mortality in subjects with T2DM, but this effect might not be due to reduced atherosclerotic events.
• Subjects with inactivating mutations in glucokinase have elevated HbA1c but no increased risk of cardiovascular disease.
Animal studies
Pros
• Effects of diabetes on myelopoiesis and atherosclerosis regression can be prevented by SGLT2 inhibitors in mice.
• Expression of aldose reductase at human levels results in exacerbated atherosclerosis in diabetic mice.
Cons
• It is difficult to know for certain if the effects of diabetes are mediated by direct effects of glucose or downstream intermediates in lesional cells.
• Several studies have failed to demonstrate a pro-atherosclerotic effect in animal models associated with clear hyperglycemia.
• Forced uptake of glucose into myeloid cells is not sufficient to produce a pro-atherogenic effect.
• SGLT2 inhibitors might act by altering hemodynamics, resulting in indirect effects on cells involved in atherosclerosis.
Isolated cells
Pros
• A large number of studies convincingly show detrimental effects consistent with pro-atherosclerotic actions of elevated glucose in endothelial cells, SMCs and immune cells.
Cons
• Cells in culture can exhibit metabolism very different from that of cells *in vivo.*
• Several cell culture studies show no effect of elevated glucose.
• Cell culture studies are difficult to control because of glucose depletion, osmotic effects, and an overabundance of energy substrates provided by most cell culture media.
• Glucose transporters have been shown to be down-regulated in the presence of elevated glucose in some cell types.