

**GLUCOSE AS A CARDIOVASCULAR COMPLICATIONS -  
OVERVIEW**

Jagdish L Kakadiya, Dr. Nehal J Shah

Department of Pharmacology, Dharmaj degree Pharmacy College,  
Petlad-Khambhat road, Dharmaj,  
Dist: Anand, Gujarat, India.



**ADDRESS FOR CORRESPONDENCE**

Mr. Jagdish L. Kakadiya  
Dharmaj degree pharmacy college,  
Amrapali Township, Petlad-Khambhat road,  
Dharmaj, Dist: Anand-388430,  
Gujarat, India.

[jagdishkakadiya@yahoo.co.in](mailto:jagdishkakadiya@yahoo.co.in)

Glucose is the driving force in microvascular complications of diabetes, yet the action of glucose alone seems inadequate and unable to account for the excess atherosclerosis observed in subjects with diabetes. In type 2 diabetes, insulin resistance, and the metabolic syndrome, the vasculature is exposed to a frontal assault by hypertension, dyslipidemia (increased triglycerides, low HDL and high LDL cholesterol), inflammation, and impaired fibrinolysis (1, 2). This toxic metabolic environment increases atherosclerotic risk in persons with the metabolic syndrome. The heightened risk observed in the metabolic syndrome inevitably sets the stage for increased vascular disease in type 2 diabetics, but hyperglycemia adds additional risk. Strong epidemiological evidence suggests a correlation among glucose, atherosclerotic plaque burden, cardiovascular events, and increased morbidity and mortality (3-5).

Although it is likely that various metabolic factors contribute to diabetic cardiovascular complications, there is much evidence to suggest that glucose plays a central role in these processes. We can find partial evidence to support this theory in clinical studies that demonstrate the benefits of glycemic control. We have already seen evidence in the previous section that suggests that conditions, which elevate blood glucose, can lead to functional changes in the cardiovascular system. Hyperglycaemic production of reactive oxygen species (ROS) what is thought to underlie cellular metabolic changes, ultimately leading to functional changes in the cardiovascular system. These functional changes in the cardiovascular system have the potential to directly affect blood pressure. Treatment outcome studies are a major piece of evidence implicating glucose as the causative agent in the development of cardiovascular alterations.

The Diabetes Control and Complications Trial (DCCT) studied the result of using an intensive treatment on the development of diabetic complication in type 2 diabetes. It was shown that the intensive treatment, as compared with a more conventional treatment regime, resulted in a daily average blood glucose level that was closer to normal, as well as reduced levels of glycosylated hemoglobin. The DCCT study also showed that the same subject with better glycemic control can improve microvascular outcomes in patient with type 2 diabetes.

The United Kingdom Prospective Diabetes Study (UKPDS) investigated the effect of a more intensive treatment for NIDDM on its related complications. Similar to the DCCT, the UKPDS found a reduction in microvascular associated pathologies in the intensive treatment group. It should be pointed out however, that the intensive treatment in the UKPDS study only

resulted in a very modest reduction in glycosylated hemoglobin levels. Significantly, the incidence of myocardial infarction, a macrovascular outcome, was 16 % lower in the intensive therapy group of the UKPDS. Taken together, these two major studies suggest that blood glucose level plays a major role in the development of both micro and macrovascular complication of diabetes mellitus. Previously it was described that vascular dysfunction is a complication associated with type 1 and type 2 diabetes. The current theory surrounding the dysfunction of vascular tissues due to further disturbances involves the production of oxygen derived free radicals, leading to further disturbances in the cell's metabolism. Furthermore, it has been reported that treatment with an oxygen free radical scavenger attenuates the impaired vasorelaxation response. Increase levels of oxidative stress have been shown in the both human diabetics, and a streptozotocin induced model of type 2 diabetes. Hyperglycemia is demonstrated to induce production of ROS in vascular endothelial cells. The production of ROS due to hyperglycemia occurs concurrently with increased protein kinase-C (PK-C) activity, advanced glycation end product (AGE) production, and flux through the polyol pathway. However, the exact nature of the cause effect relationship between these elements is still not clear.

The mitochondrial electron transport chain is thought to be the source of the excess generation of ROS due to hyperglycemia. Nishikawa et al; 2000 demonstrated that inhibiting mitochondrial production of ROS would inhibit hyperglycemia induced increase in PK-C activity, AGE production and sorbitol accumulation. This would suggest that the production of ROS precedes the induction of PK-C, the formation of AGEs, and the accumulation of sorbitol. Unfortunately, the pathways do not seem to be that simple. Inoguchi et al suggest that the formation of ROS occurs due to hyperglycemia activation of PK-C dependent reduced Nicotinamide adenine dinucleotidephosphate (NADPH) oxidase. Although it seems that these two references may contradict each other, it is entirely possible that both results are true. For instance, it seems that the production of AGEs can be initiated by ROS production, but formation of AGEs has also been demonstrated to result in the production of ROS. Regardless of the precise mechanism of hyperglycemia induced ROS production, the phenomenon has been well demonstrated. Furthermore, we know that the overproduction of ROS has definite downstream consequence on the function of cardiovascular tissues.

The involvement of ROS has been implicated in vascular system. One model in which ROS, specifically superoxide, has proposed to lead to after nitric oxide synthase (NOS) co factor

tetrahydrobiopterin. Once uncoupled by deactivation of its co factor, NOS will also begin to produce oxygen derived free radicals such as superoxide anion and hydrogen peroxide. The end result of peroxynitrite is the decreased level of NO, and an increased production of oxygen derived free radical. Subsequently, the end result of reduced NO level would be an impairment of the endothelium dependent relaxation of vascular tissue. ROS generated under these circumstances have also been shown to lead to the further alteration of metabolic pathways including activation of PK-C, production of AGEs, and induction of the polyol pathway. Although all of the three pathways have been identified to lead to diabetic complication, sorbitol accumulation via the polyol is not implicated in the development of cardiovascular complications per se. the induction of PK-C will lead to increased contraction of vascular smooth muscle. Increased PK-C activity has also been associated with proliferation of vascular endothelium and smooth muscle, increased vascular permeability, and induction of expression of endothelin-1 (ET-1). The activation of AGEs has been shown to disrupt both intracellular and extracellular matrix proteins, generate free radicals via interaction with its receptor, interfere with NO levels, and stimulate the production of ET-1. The induction of PK-C and production of AGEs provide a further molecular basis for the dysfunction of cardiovascular tissues, and an alteration of blood pressure. An attenuated level of NO production, induction of PK-C, and production of AGEs, provide, at least in part, a metabolic basis for altered reactivity in vascular tissues. Although we have focused this discussion on how these metabolic changes would likely affect vascular tissues, there is evidence that suggest that similar metabolic disturbance occurs in tissues not sensitive to insulin, such as Cardiomyocyte. This may suggest that vascular tissues are metabolically sensitive to extracellular glucose concentration in a way similar to non- insulin dependent type tissues.

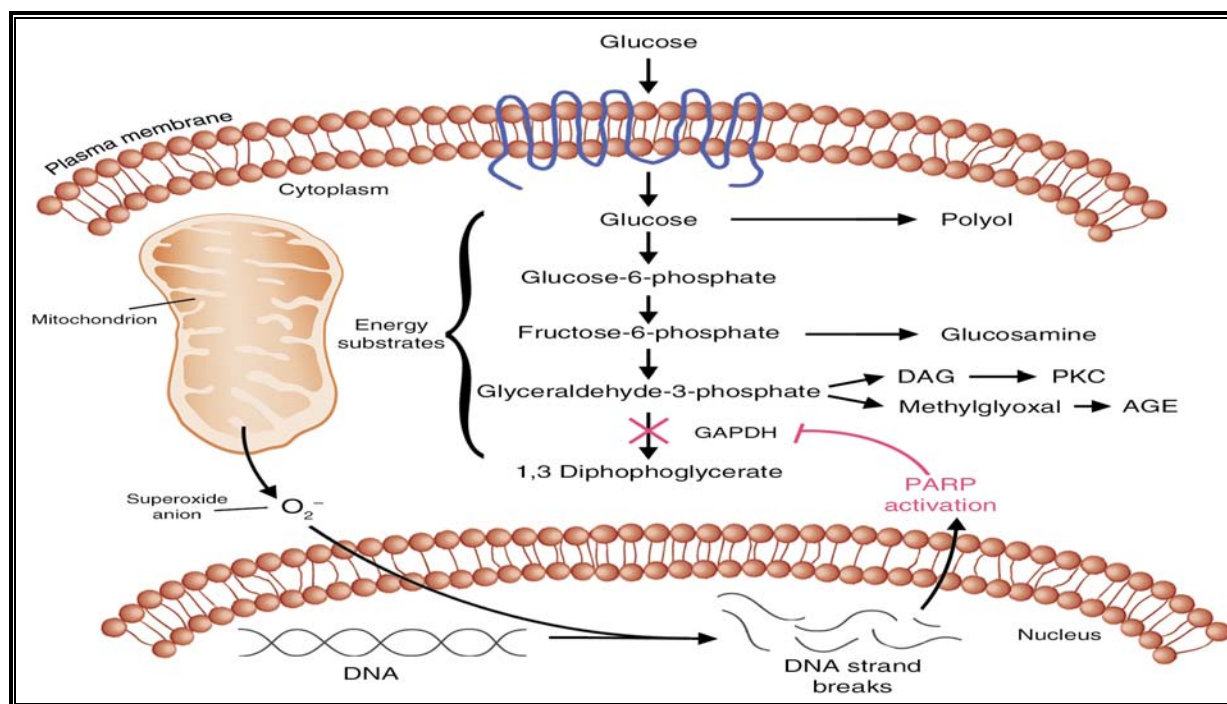
In an autopsy study of 18- to 34-year-olds there was an increase in atherosclerotic plaque burden in subjects with elevated hemoglobin A1C (5). The Honolulu Heart Program demonstrated a predictive correlation between fasting plasma glucose levels (nondiabetic, impaired glucose tolerance, and diabetic ranges) and cardiovascular events and mortality (6). Large population studies from Northern Europe indicate a direct correlation between glycemic control (as measured by glycohemoglobin) and cardiovascular morbidity and mortality (4, 7). As will be discussed later, hyperglycemia has specific deleterious effects upon vascular endothelial function that could account for these epidemiological correlations between hyperglycemia and

poor vascular outcomes. One would predict, based upon epidemiological data, that interventional studies targeting hyperglycemia would show improved cardiovascular outcomes. To date, no such compelling evidence has emerged. In fact, in the largest prospective glucose-lowering trial in type 2 diabetes patients, the United Kingdom Prospective Diabetes Study (8), there were no statistical improvements in cardiovascular outcomes when glucose was lowered using insulin or sulfonylureas. Only in the small metformin cohort ( $n = 342$ ) were cardiovascular outcomes improved by optimal glycemic control. A few small studies have suggested a positive impact of glycemic control on cardiovascular events, but this point remains highly debated. The conclusions of a recent panel convened by the American Heart Association suggest that while glycemia contributes to cardiovascular risk, treatment of glycemia, exclusive of other potent cardiovascular risk factor intervention, is inadequate to reverse or reduce the complex atherosclerotic process (6).

### **MECHANISM OF GLUCOTOXICITY**

The negative impact of hyperglycemia on endothelial function and patho logical changes observed in diabetes is supported in the literature (9, 10). Endothelial cells in vitro are exquisitely sensitive to high glucose (25mM). Nishikawa et al. (10) and others have carefully characterized four major molecular signaling mechanisms activated by hyperglycemia in endothelial cells and other cell types vulnerable to hyperglycemic injury. These include activation of PKC (via diacylglycerol), increased hexosamine pathway flux, increased advanced glycation end product (AGE) formation, and increased polyol pathway flux. Nishikawa et al. recently proposed the existence of a unifying mechanism that integrates the above pathways: increased production of reactive oxygen species (ROS) (specifically superoxide) by the mitochondrial electron transport chain (10).

In their original report, numerous theoretical constructs were outlined for the impact of altered redox state upon formation of polyols, AGEs, and PKC. What remained unclear were the downstream targets of oxidant stress. In the paper by Du et al. (14) in this issue of the *JCI*, this group takes their seminal observation one step further and defines one consequence of increased ROS, namely activation of PARP. PARP activation leads to ribosylation and inactivation of GAPDH. Inhibition of GAPDH increases delivery of glycolytic intermediates to the mitochondria. This change in turn would be expected to increase mitochondrial superoxide production and also to increase flux through the AGE and PKC glucotoxic pathways (Figure 1).



**Figure 1** High glucose flux through constitutive glucose transporters on endothelial cells overwhelms the mitochondrial electron transport system. Excess mitochondrial substrate flux results in the generation of reactive oxygen species that cause DNA strand breaks and activation of PARP. PARP ribosylates and inactivates GAPDH, thereby disrupting normal glucose metabolism. Inactivation of GAPDH effectively shunts glucose into the polyol pathway and leads to activation of PKC and accumulation of AGEs and glucosamine. DAG, diacylglycerol.

### MECHANISMS OF HYPERGLYCAEMIA INDUCED VASCULAR DAMAGE

In addition to insulin resistance, hyperglycaemia itself can also induce cellular dysfunction via multiple intermediates and their downstream effectors. It has been known for some time that exposure to glucose leads to accumulation of glycolytic metabolites and glucose modified proteins that are known exclusively as glucotoxins under certain circumstances. In general, the adverse effects of hyperglycaemia can cause vascular dysfunction either by generating toxic and reactive metabolites or by altering intracellular signalling pathways. Several theories have been proposed and include the AGE theory, the PKC theory and the reactive oxygen intermediate theory. They control the fate of cells by interacting with intracellular transcription factors as well as by controlling the expression of secreted growth factors and cytokines. Identification and an understanding of these factors are essential to the development of pharmacological interventions.

### **The advanced glycation end-product theory**

Glycation of many structural and intracellular proteins occurs through covalent and cross-linking modifications by glucose, which may change protein conformation and permanently impair their functions (11). AGEs can also alter cellular functions by binding to their receptors, such as the receptor for AGEs (RAGE) or other receptors, including the macrophage scavenger receptor, p60, p90 and galectin-3. RAGE is a transmembrane protein that belongs to the immunoglobulin family (12). Upon binding to AGE-modified proteins, RAGE initiates multiple cascades of cellular signalling pathways, including p44/42 MAPK and PKCs, and further disrupts cellular homeostasis (12).

### **The reactive oxygen species theory**

Hyperglycaemia is known to induce oxidative stress. Oxidative phosphorylation of glucose in mitochondria generates free radical byproducts, including superoxide anion, production of which increases with the hyperglycaemia (13). Free radicals damage cellular proteins (14) as well as mitochondrial DNA (15), and can also confer profound effects on cell biology through their effects on multiple effectors. For example, increased oxidative stress downregulates NO levels (16, 17), and promotes leucocyte adhesion to the endothelium and disrupts its barrier function (18). Oxidative stress may also alter signalling pathways, such as the PKC pathway, that are intrinsic to cellular functions.

### **The protein kinase C theory**

PKC is a family of enzymes comprised of at least 12 members (19) that are classified as conventional PKC, novel PKC and atypical PKC. PKCs are intracellular signalling molecules that can regulate diverse cardiovascular functions, including vascular permeability, vasodilator release, endothelial activation, cardiomyocyte contractility and growth factor signalling. Receptor mediated activation of PKC occurs through the activation of phospholipase C, whereas pathological activation of PKC can occur in diabetes. Elevated glucose levels will enhance glycolytic pathway flux in the diabetic state and increase intracellular glyceraldehyde-3-phosphate, which in turn upregulates diacylglycerol and activates PKC (20).

Levels of diacylglycerol and PKC activation are increased in various tissues in diabetic animal models (21). Experimental diabetic rats have elevated PKC activities in aorta and heart, especially beta and delta isoforms. PKC activation in blood vessels of the retina, kidney and nerves can produce vascular damage, including increased permeability (22), NO dysregulation

(23, 24), increased leucocyte adhesion (25) and alterations in blood flow (26, 27). PKCs may confer isoform-specific effects on the homeostasis of cardiomyocytes. Targeted over-expression of PKC-beta-2 in mouse myocardium resulted in severe ventricular hypertrophy, interstitial fibrosis, cardiomyocyte necrosis and impaired contractility (28, 29) reminiscent of diabetic cardiomyopathy. PKC-epsilon translocation is detected during cardiac pre-conditioning and confers cardioprotection against ischaemia/reperfusion injury, whereas activation of PKC-delta exacerbates the damage (30, 31). PKC activation may also increase the expression of various growth factors from cardiomyocytes and vascular cells. For example, expression of VEGF (32), transforming growth factor-beta (33, 34), endothelin-1 (35) and connective tissue growth factor (36), as well as their signalling (37), are all regulated by PKC. These factors play key roles in the maintenance of cardiovascular homeostasis. In addition, PKC activation can also activate pivotal signalling pathways such as Erk, nuclear factor-kappa B (38) and retinoblastoma protein (39) that regulate cell cycles and further determine cell proliferation, growth and death.

#### **Cross-talk between these theories**

These pathways cross-talk extensively, resulting in a sophisticated network that ensures that normal cell functions continue. This is not surprising because metabolites of glucose are substrates or cofactors for many metabolic pathways (40). The clearest example of interactions among the different pathways is the formation of oxidants. Elevated levels of oxidants may be derived from increased mitochondrial superoxide production in the diabetic state. Some AGEs are oxidants and contribute to glycooxidation and lipid peroxidation (41). AGE binding to RAGEs can also increase oxidant production by stimulating NADP (H) oxidase (42). Oxidant generation by the myeloperoxidase system of phagocytes and by mitochondrial metabolism can stimulate the production of intracellular AGEs (43). Activation of PKC can affect the production of oxidants and AGEs by activation of oxidases such as NADP (H) oxidase (44). Both AGEs and oxidants can induce PKC activation and further impair normal cell functions (45, 46).

A clear understanding of the interaction among these theories is critical for designing therapeutic strategies. The multiplicity of pathways by which hyperglycaemia can generate toxic metabolites probably explains the general lack of efficacy of interventions targeted at a specific glucotoxin, which is therefore insufficient to prevent or reverse diabetic complications. The common downstream intermediates and effectors appear to be logical targets.



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