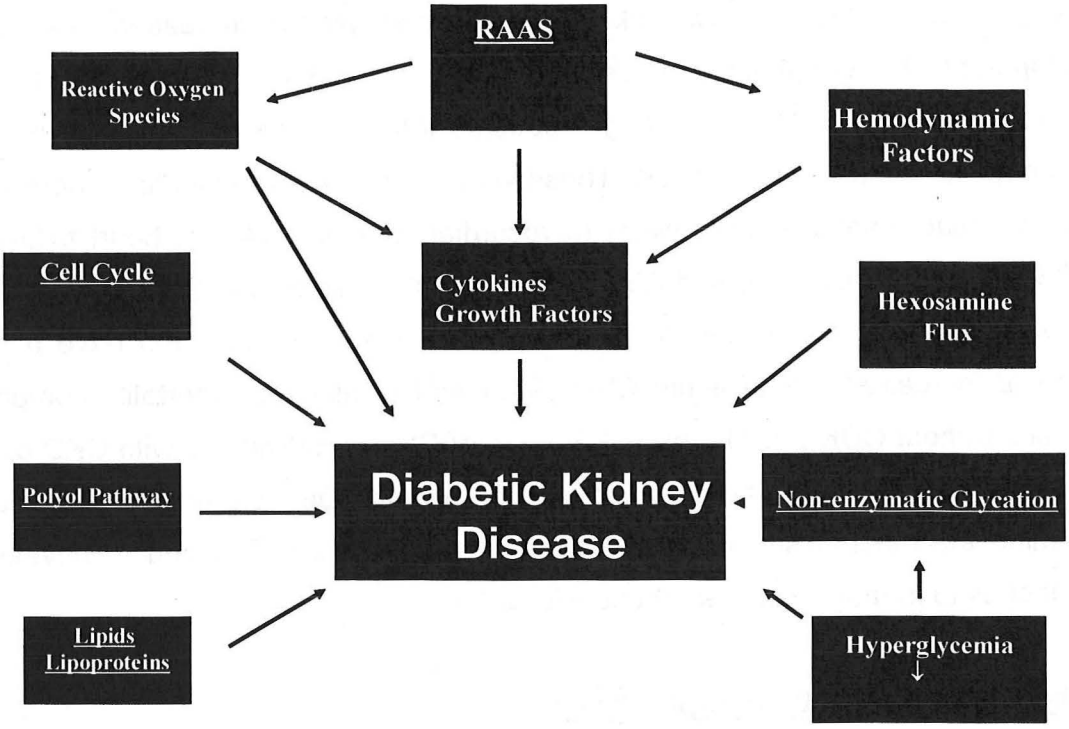
Diabetic Kidney Disease:

Emerging Mechanism, New Therapeutic Possibilities



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Background

In developed nations, diabetes mellitus (DM) is the leading cause of microvascular disease like chronic kidney disease (CKD) and end-stage kidney failure (ESKD) (United States Renal Data System, USRDS 2003 Annual Report). CKD is a harbinger for future cardiovascular disease because it increases the risk for development and progression of cardiovascular disease (CVD) and for death from cardiovascular causes (1). Accordingly, patients with CKD are considered the "highest risk group" for subsequent CVD (2). Those with CKD are two to five time more likely to suffer an acute cardiovascular event (myocardial infarction, stroke, heart failure) than unaffected individuals (3;4), and ESKD increases this risk to greater than twenty times that of the general population (4;5). Patients with diabetes and CKD have a roughly three-fold increased risk of acute CVD event and of all-cause mortality compared to diabetics without CDK (6). The excess burden of CVD in individuals with CKD or ESKD necessitates ongoing efforts to a) understand the pathogenesis of both conditions, b) determine the mechanism underlying the observed associations and c) develop new approaches to manage and treat those with CKD.

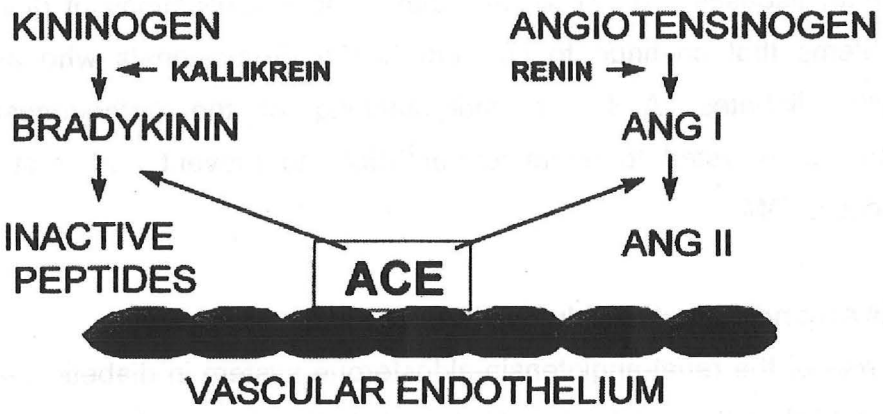
Mechanisms of Diabetic Complications

. The Diabetes Complications and Control Trial highlighted the importance of effective glycemic control in preventing microvascular complications like nephropathy and retinopathy in persons with T1DM (7). Long-term follow-up of DCCT participant (the EDIC study) have validated the complication-related benefits of strict glycemic control (8;9), but these studies also suggest that hyperglycemia alone is not sufficient to account for all of the complications; about 40% will develop kidney and or retinal disease and approximately 50% will develop coronary artery disease (7-9). Similarly, the United Kingdom Prospective Diabetes Study (UKPDS) of type 2 diabetic subjects demonstrated that in this population, proper metabolic control predicted favourable long-

term outcome with respect to development and progression of micro- and macrovascular diseases (10-12). Nevertheless, the complications of diabetes remain vexing problems that continue to confront health professionals who are managing patients with diabetes. A better understanding of the pathogenesis of these complications is expected to enhance our ability to prevent and treat the adverse consequences of DM.

a) i) The RAAS and Diabetic Kidney Disease

The role of the renal-angiotensin-aldosterone system in diabetic glomerulopathy has been established through basic and clinical studies (13-15). Angiotensin II regulates renal hemodynamics through its influences on glomerular efferent and afferent arterioles, and on systemic blood pressure (13;16) . In addition, angiotensin II manifest important non-hemodynamic effects on the kidney including, but not limited to:- i) increased matrix synthesis; ii) upregulation of many cytokines including transforming growth factor beta 1 (TGFβ1), connective tissue growth factor (CTGF) and plasminogen activator inhibitor 1 (PAI-1) that are key mediators of extracellular matrix biology; iii) induction of several inflammatory cytokines including interleukin (e.g IL-6) and vascular cell adhesion molecule 1 (VCAM-1). Accordingly, treatment with angiotensin II converting enzyme inhibitors (ACEi) or an angiotensin II receptor antagonist (ARBs) is now the standard of care for diabetic kidney disease. In animal studies, overexpression of renin, the enzyme responsible for generation of angiotensin I from angiotensinogen causes severe renal and cardiovascular disease that is attenuated by angiotensin II type 1 receptor blockers (17;18). Recent clinical studies like the HOPE trial involving those with diabetes further emphasize the importance of the RAAS in progression of diabetic micro- and macrovascular disease (14). However, as is the case for metabolic control, many affected individuals who receive ACEi and / or ARBs will experience progression of kidney disease suggesting the existence of other processes that influence disease development.



RAS and kallikrein-kinin system

Circulation. 97:1411-1420, 1998

ii) Is Bradykinin important?

While ACEi administration are current recommended pharmacologic agents for treating individuals with diabetic kidney disease, there is some evidence that the efficacy of these agents is not only related to blockade of the harmful consequences of RAAS over-activity, but to the accumulation of bradykinin that occurs secondary to ACE (Kininase) inhibition.(19-21) For instance, a polymorphism in exon 1 of the bradykinin B2 receptor has been associated with lower albumin/creatinine ratio (22;23). In animal studies, diabetic Bradykinin B2 receptor mice developed more severe albuminuria and glomerulosclerosis compared with diabetic non-target littermates (24). These studies are relatively recent and limited in number; hence, a role for kinin in diabetic kidney disease remains controversial.

b) Protein Kinase C (PKC) and Diabetic Kidney Disease

Protein kinase C catalyzes serine or threonine phosphorylation of protein substrates to mediate an array of cellular processes in a cell-specific manner. PKC may be activated by inositol 1,4,5-triphosphate (IP3), that is generated from G-protein-coupled hydrolysis of phosphatidylinositiol 4,5 bisphosphate to IP3 and diacylgerol (DAG). DAG generated through phosphatidylcholine (PC) hydrolysis may also activate PKC. *De novo* DAG synthesis from glucose is believed to be the mechanism through which glucose activates PKC under hyperglycemic conditions. This has been demonstrated to occur in mesangial, vascular smooth muscle and endothelial cells, as well as in glomeruli of diabetic rats (25-28).

The PKC isoform activated in diabetes is dependent on the tissue type; in the case of renal glomeruli, hyperglycemia and diabetes activate isoforms PKCα and PKCβ1 (27;28). PKC activation may mediate increased oxidative stress via activation of NAD(P)H oxidase and generation of superoxide. In fact, administration of a PKC beta isoform-specific inhibitor (LY333531) to diabetic rats alleviates glomerular oxidative stress. Furthermore, LY333531 also improved albumin excretion rate (AER) and glomerular filtration rate (GFR) in this model (27;28). A phase II clinical trial of an oral formulation of the aforementioned PKC beta inhibitor (ruboxistaurin, RBX) has been completed in individuals with type 2 diabetes mellitus (29). The patients were type 2 diabetics with proteinuria, plasma Cr 1.7 mg/dL (women) to 2.0mg/dL (men) who were being treated with either an ACEi or ARB or both and who received multiple risk factor intervention for metabolic and blood pressure control, After 1 year, RBX treatment caused a 24% decrease in ACR compared to a 9% decrease in the control group (29). It thus appears that RBX may be a beneficial add-on therapy in type 2 diabetic patients with proteinuria.

c) Advanced Glycation Endproducts (AGES)

Advanced glycation endproducts (AGEs) are compounds that may be formed in routine physiologic processes, but whose production is accelerated in various

pathophysiologic conditions including hyperglycemia, oxidative stress, inflammation and chronic diseases (like chronic kidney and neurodegenerative diseases). The precise mechanisms leading to AGE formation have not been completely elucidated. The early stage is characterized by non-enzymatic reaction of an amino group of a protein with glucose to form a Schiff base, followed by Amadori rearrangement and latter by several reactions eventually terminating in the formation of AGE structures like carboxymethyllysine (CML) and pentosidine (30-33).

AGE-modified adducts of proteins (like CML) serve as markers of AGE formation under various conditions in human and animal models. These AGE-modified proteins mediate various adverse reactions including macrophage activation, increased cytokine production, matrix metalloproteinase activation and matrix derangement (33-36). In addition, AGE formation leads to increased cross-linking of matrix proteins further disrupting protein-protein and protein-cellular interaction in matrix (33;34). Similarly, non-matrix protein may be AGE-modified, resulting in abnormal cellular responses and deranged physiology. Importantly, AGE may interact with and bind to receptors for AGEs, known as RAGE, as well as to other receptors including type II macrophage scavenger receptor (32;36;37). In addition to possessing strong collagen crosslinking activities, in humans, serum AGEs correlate with DM complications (retinopathy, nephropathy and atherosclerosis), increase vascular injury and permeability, may be procoagulant, promote monocyte influx / migration and may interfere with NOS / NO production. As a result, investigations have been undertaken over the past 10 to 15 years to determine whether prevention of AGE formation would impact on the aforementioned adverse consequences of hyperglycemia, oxidative stress and inflammation.

Two approaches have been employed to mitigate the harmful effects of AGE-protein adducts. The first utilizes blockers of AGE formation, while another more recent strategy involves administration of compounds that are capable of interrupting previously-formed AGEs ('AGE breakers').

Aminoguanidine (Pimagidine), an AGE inhibitor has been studied in type 2

diabetic humans with CKD in the ACTION trial. ACTION I and II were double-blind, placebo-controlled, randomized clinical trials designed to evaluate the safety and efficacy of aminoguanidine in retarding the rate of progression of renal disease in participants with overt diabetic nephropathy. Participants who were taking ACEi (76%) and lipid-lowering agents (43%) were maintained on these regimens through the study and the primary endpoint in the study was time to doubling of baseline creatinine. In ACTION I, there was no statistical significant difference between the placebo and aminoguanadine group with respect to the primary outcome. However, there was a trend to slower progression in the latter group and this group also manifested statistically lower LDL-cholesterol, triglyceride and urine protein. The follow-up study, ACTION II was terminated early due to lack of efficacy and safety concerns (38). Other AGE inhibitors being considered for treatment of diabetic kidney disease include OPB-9195 and ALT-946.

Tthe alternate approach of using AGE breakers is currently being investigated. In animal studies, the AGE breaker ALT-711 (Alagebrium) inhibits PKC isoform phosphorylation and nuclear translocation, proteinuria and renal fibrosis (39-41). Clinical trials in human, in which the drug was being evaluated for treatment of systolic hypertension and erectile dysfunction have been halted because of safety concerns and lack of a treatment effect.

The last aspect of AGE pathobiology and diabetic nephropathy has focused on investigation of RAGEs. Ligands for these receptors include N-epsilon (carboxymethyl) lysine (CML) and S100/Calgranulin. Interaction between RAGE and its ligands upregulates the receptor and leads to a number of downstream effects including increased expression of vascular endothelial growth factor (VEGF) in podocytes and recruitment of mononuclear cells to the glomeruli (32;34;35). In RAGE overexpressing transgenic mice, treatment with soluble RAGE (sRAGE) diminishes proteinuria and glomerulosclerosis and improves renal function (42); sRAGE captures circulating AGE and prevents these ligand from binding to cell surface receptors. Therefore, this approach provides a potential alternative strategy for managing diabetic kidney disease

in the future.

d) Hexosamine Pathway Flux

In the basal state, only a small proportion of the glucose that enters cells (estimated at 1-3%) is shunted through the hexosamine pathway. In this pathway (Figure 2), glucose-6-phosphate is converted to glucosamine-6-phosphate via the action of the rate-limiting enzyme glutamine:fructose-6-phosphate amidotransferase (GFAT). GFAT may be a key factor that regulates the effects of the hexosamine pathway on cellular processes (43). It is believed that, by acting as a sensor of glucose levels in the extracellular milieu, the hexosamine pathway may participate in regulating the cellular responses to changes in glucose concentration in the extracellular compartment (43-45).

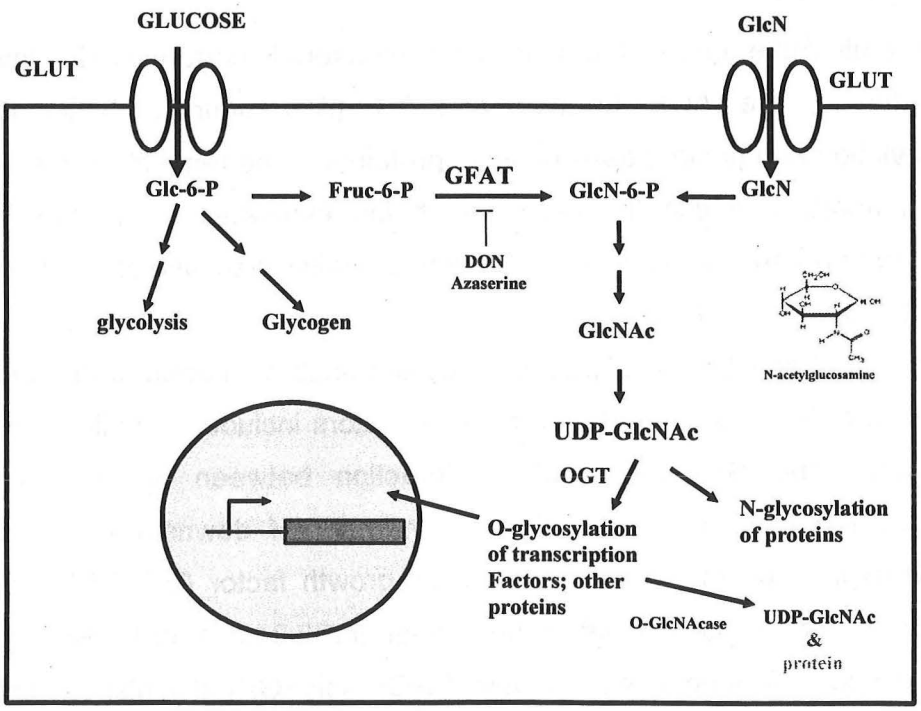


Figure 2: The Hexosamine Pathway and O-glycosylation

A key product of this pathway, uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), is the substrate for the N- and O-linked glycosylation of intracellular proteins. The intracellular levels of O-linked glycosylated proteins correlate with GFAT activity, and blockade of GFAT activity, or inhibition of expression of GFAT with antisense oligonucleotides, lowers intracellular levels of O-GlcNAc-modified proteins. Some studies support a link between the hexosamine pathway and the development of insulin resistance (43-46). In addition, the hexosamine pathway also influences the expression of the genes for growth factors such as transforming growth factor α (TGFα) and TGF beta 1 (TGFβ) (45-49).

Hexosamine Pathway Flux and tissue injury: Relationship to Diabetic Glomerular Disease?.

In humans, investigators have compared GFAT protein expression in normal kidneys and in kidneys from type I diabetes mellitus patients with clinical nephropathy. Immunostainable GFAT was present at low levels in normal glomeruli, while diabetic glomeruli exhibited increased GFAT expression in both glomerular epithelial and mesangial cells(50). Although a link between GFAT activity and diabetic glomerular injury was not established by this study, previous reports indicated that glucose-induced increases in TGFß1 expression and activity in porcine mesangial cells are mediated by glucose flux though the hexosamine pathway (51). These studies suggest that GFAT expression is upregulated by hyperglycemia and the diabetic state and that glucose flux through the hexosamine pathway may be an important determinant of diabetic glomerular injury.

In line with of these observations, recent studies in humans support a link between polymorphism in GFAT genes (*GFPT1* and *GFPT2*) and diabetic nephropathy. IN African Americans, but not Caucasians, a polymorphism in the 3' untranslated region (3'UTR) of *GFPT1* may associate with diabetic nephropathy (52). In a related study,

variants in the 3'UTR and exon 18 of GFPT2 were associated with type 2 diabetes in Caucasians and with diabetic nephropathy in African American (53). Further investigations will be required to clarify the role of this nutrient pathway in diabetic complications.

e) Reactive Oxygen Species

Hyperglycemia has been associated with the generation of reactive oxygen species (ROS), and some of the complications of DM may be related to oxidative injury (54;55). Mesangial cells exposed to high ambient glucose conditions manifest increased ROS like hydrogen peroxide, superoxide anion and hydroxyl radicals (56;57). Likewise, excess flux through the polyol pathway may serve as a source of ROS generation due to depletion of cofactors like NADH and NAD(P)H; ROS may also be producing during the regeneration of these factors by NAD(P)H oxidase. Accordingly, NAD(P)H oxidase may generate reactive intermediaries like superoxide and hydrogen peroxide that increase cellular oxidative stress (54;58).

ROS are believed to promote tissue injury through multiple mechanisms including:- membrance and intracellular lipid peroxidation, increased extracellular matrix production, enhanced inflammatory cytokine generation and increased expression of mediators like TGFβ-1. However, it should be noted that the precise mechanisms that lead to adverse consequences of elevated cellular ROS levels are incompletely understood. Nonetheless, the important pathophysiologic consequences of ROS are highlighted by the existence of multiple, redundant processes that serve to protect cells against these reactive intermediary metabolities. Included amongst these are:- i) cytosolic super oxide dismutase (CuZn-dependent, CuZnSOD) and mitochondrial super oxide dismutase (Mn-dependent, MnSOD) that catalyzes the conversion of superoxide to hydrogen peroxide; ii) cytosolic and peroxisomal catalase that converts hydrogen peroxide to water; iii) glutathione perosxidase (cytosolic and perosxisomal) which breaks down hydrogen peroxide. In this regard, overexpression of SOD protect mice against diabetic complications including kidney disease (59;60).

The naturally occurring anti-oxidant, lipoic acid, serves a key role in regulating the redox status of cells primarily by enhancing synthesis of key molecules such as glutathione (61;62). Accordingly, treatment with alpha lipoic acid has shown a positive impact on improving and preventing diabetic polyneuropathy (61;63;64), its influence on other complications such as diabetic nephropathy is unknown and has not been extensively tested, but some animal studies suggest a potential benefit of this intervention (65;66).

f) Unified Hypothesis of Diabetic Complication – Brownlee's Hypothesis

Recently, a '*unifying hypothesis*' of hyperglycemia mediated complications has been proposed; it is based on the generation of reactive oxygen species in the mitochondria and suggest that effective blockade of ROS generation could be of therapeutic benefits (67;68).

The central feature of this unified hypothesis is based on the observation that hyperglycemia inhibits the enzyme glyceraldehye 3-phosphate dehydrogenase (GAPDH) activity through the generation of superoxide in the mitochondrion (69). GAPDH inhibition leads to a) accumulation of glyceraldehye 3-phosphate which enhances AGE formation through increased formation of the AGE precursor methylglyoxal and b) PKC activation by DAG that is generated from glyceraldehye 3-phosphate. Accumulation of the upstream metabolite fructose-6 phosphate enhances hexosamine pathway flux and finally, the buildup of glucose further upstream increases polyol pathway flux and sorbitol generation (Figure 3).

In support of this hypothesis, overexpression of MnSOD that degrades hydrogen peroxide or uncoupling protein 1 (UCP-1) which collapses mitochondrial voltage gradient prevents hyperglycemia-induced ROS generation (70). In endothelial cells, inhibition of GAPDH activity by antisense oligonucleotide (ODN) leads to activation of PKC, increased AGE formation and enhanced flux through the hexosamine pathway (71). The observation that hyperglycemia inhibits GAPDH by modifying this enzyme with ADP-ribose polymers, raised the question of whether hyperglycemia activated poly-

ADP ribose polymerase (PARP), a nuclear- resident DNA repair enzyme (71). ROS

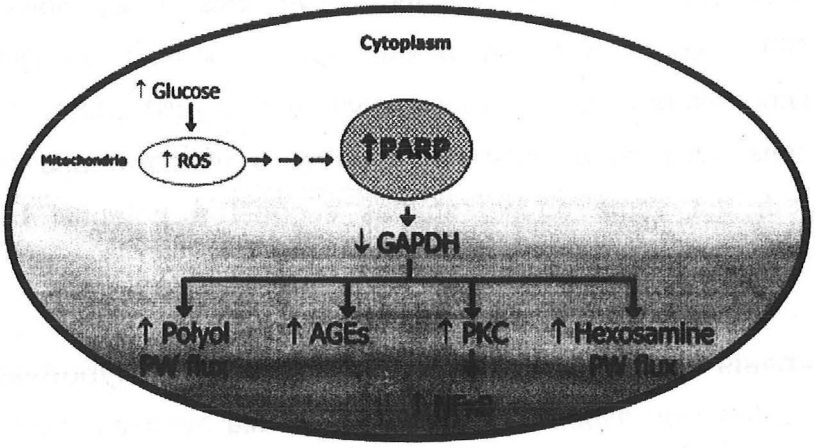


Figure 3: Unifying mechanism of hyperglycemia-induced cellular damage (***Diabetes* 54:1615, 2005**)

cause DNA damage (strand breaks), PARP is activated to repair DNA and does so through generation of ADP-ribose from NAD^+. Subsequent generation of polymers of ADP-ribose inhibits GAPDH as it shuttles between nucleus and cytoplasm to participate in the DNA repair process (56;71).

Thiamine (Vitamin B1), the Pentose Phosphate Pathway and Diabetic Complicationn

Fructose-6 phopshate and glyceraldehydes 3-phosphate are products of the non-oxidative branch of the pentose phosphate pathway that are produced by a thiamine-dependent enzyme, transketolase. The overall direction of transketolase catalyzed generation of these two metabolites is driven by substrate concentration. Hyperglycemia increases fructose-6 phopshate and glyceraldehydes 3-phosphate, and subsequently increases flux through the polyol, AGE and hexosamine pathways and activates PKC (Figure 4) (72).

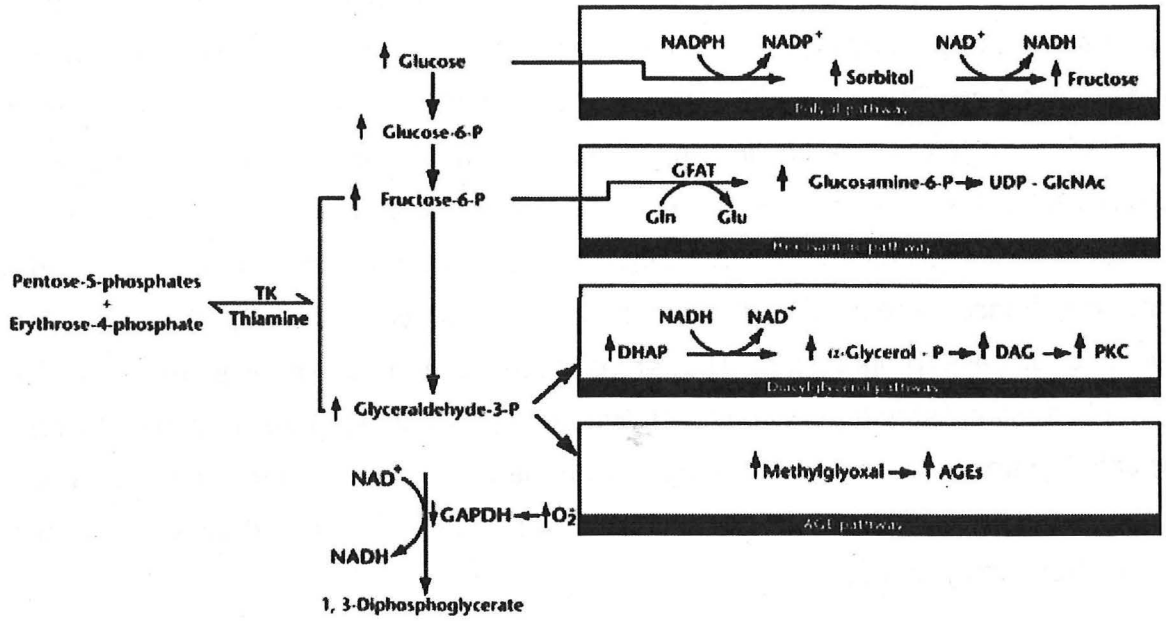


Figure 4: Depiction of possible mechanism whereby Benfotiamine may inhibit hyperglycemic complications (72).

Benfotiamine is a lipid-soluble analogue of thiamine with greater bioavailability (73). Recent observations suggest that benfotiamine is capable of blocking major pathways that mediate adverse consequences of hyperglycemia through activation of transketolase (72). Benfotiamine seems to be beneficial in the treatment of diabetic polyneuropathy in human and animal studies (74;75), and in combination with thiamine may prevent diabetic nephropathy in rats (76). Further clinical trials to test the efficacy of thiamine and benfotiamine for the prevention of diabetic complications in humans are currently in progress.

Summary

Diabetes Mellitus and its complications represent a growing social and financial burden throughout the world. Given the influence of kidney disease on cardiovascular disease and mortality, additional efforts and resources will be needed to achieve the

goals of a) understanding disease mechanisms and its relationship to cardiovascular disorders and b) preventing development and progression of kidney disease. Landmark studies like the DCCT, EDIC, UKPDS and HOPE have clearly highlighted the importance of good metabolic and blood pressure control in the reduction of complication. However, in all of these studies, metabolic and blood pressure controls do not abolish complications. Other genetic and environmental factors seem to be important, hence necessitating the search for novel processes and pathways that may influence disease progression. The areas covered in this overview reflect ongoing efforts to accomplish the goals outlined. Better understanding of disease mechanisms is expected to translate into novel therapies and management approaches and to reduced social and economic burden of DM and its complication on affected individual or those at risk, their family and society.

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