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Preservation of renal function in chronic diabetes by enhancing glomerular glucose metabolism

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Abstract

Diabetic Nephropathy (DN) affects approximately 30–40% of patients with type 1 (T1DM) and type 2 diabetes (T2DM). It is a major cause of end-stage renal disease (ESRD) for the developed world. Hyperglycemia and genetics are major causal factors for the initiation and progression of DN. Multiple abnormalities in glucose and mitochondrial metabolism induced by diabetes likely contribute to the severity of DN. Recent clinical studies in people with extreme duration of T1DM (>50 years, Joslin Medalist Study) have supported the importance of endogenous protective factors to neutralize the toxic effects of hyperglycemia on renal and other vascular tissues. Using renal glomeruli from these patients (namely Medalists) with and without DN, we have shown the importance of increased glycolytic flux in decreasing the accumulation of glucose toxic metabolites, improving mitochondrial function, survival of glomerular podocytes, and reducing glomerular pathology. Activation of a key glycolytic enzyme, pyruvate kinase M2 (PKM2), resulted in the normalization of renal hemodynamics, mitochondrial and glomerular dysfunction, leading to the mitigation of glomerular pathologies in several mouse models of DN.

Keywords

diabetic nephropathy; glucose metabolism; glycolysis; PKM2; metabolites

Introduction

Diabetic nephropathy (DN) affects approximately 30–40% of people with type 1 (T1DM) and type 2 diabetes (T2DM)[1–3]. It is the leading cause of end-stage renal disease (ESRD) in the United States and Europe. Effective treatments for ESRD are dialysis and kidney transplantation in the United States. More than 100,000 people start dialysis each year and 200,000 patients with ESRD are due to DN[4]. Further, the mortality rate is over 70% within 5 years after the initiation of dialysis. Recent increases in the prevalence of T2DM, especially with early onset of the disease, can potentially elevate the number of people with

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ESRD. There is a great need for precise understanding of the pathogenesis of DN as well as new therapies to prevent and intervene its development and progression.

The risk factors for the development of DN are hyperglycemia, duration of diabetes, hypertension, and possibly insulin resistance. Intervention studies in Diabetes Control and Complications Trial (DCCT) for T1DM and the United Kingdom Protective Study (UKPDS) for T2DM, and many other studies have clearly shown that the progression of DN can be decreased with the improvement of glycemic control[5–7]. Further, inhibitors of angiotensin actions have been shown to delay the progression of DN as measured by serum creatinine doubling and the rate of progression to dialysis[8, 9]. However, it is unclear whether angiotensin-converting-enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARB) can reduce the pathology of DN[10]. In addition, blood pressure control is clearly important, especially for T2DM. Recent exciting clinical outcomes using sodium-glucose cotransporter 2 (SGLT2) inhibitor, which inhibited SGLT2 in renal proximal tubules and enhance glycosuria, appear to delay the progression of DN, possibly by multiple mechanisms including the improvement of glycemic control and insulin sensitivity, decreases in blood pressure and body weight and alteration in glomerular and tubular hemodynamics[11]. However, whether SGLT2 inhibitors can delay the progression of glomerular and tubular pathology induced by diabetes remain unclear.

Intensive studies on the potential mechanisms by which hyperglycemia could possibly cause glomerular and tubular abnormalities in diabetic patients have provided multiple hypothetical pathways including increased flux in aldose reductase pathways[12], elevated formations of advanced glycation end product[13, 14], increased levels of inflammatory cytokines[15] and excessive oxidative stress[16]. Further, diabetes induced renal toxicity caused by elevated uric acid and enhanced actions of fibrotic factors such as TGF β and CTGF have been reported[17, 18]. In addition, dysregulations of signaling pathways are reported to be associated with hyperglycemia and diabetes such as the activation of protein kinase (PKC) and other stress kinases (p38MAPK), which in turn can induce a variety of glomerular and tubular pathologies[19, 20]. However, clinical trials using inhibitors of aldose reductase pathways, antioxidants, anti-inflammatory agents, inhibitors of PKC β , advanced glycation end products (AGEs) and their receptors, and anti-fibrotic cytokines fail to show robust responses to delay or prevent the progression of DN in the presence of hyperglycemia. The risk factors of DN and their potential causal theories have been well described. In this review, we will summarize the recent progress on the molecular pathogenesis of diabetic microvascular complications with major focus on the role of glucose dysregulation and its normalization to delay the progression of DN.

Mounting evidences have demonstrated that diabetic vascular complications are the final result of two groups of counteracting factors (Fig. 1). One group manifests the toxic effects of hyperglycemia. Their counteracting factors are those endogenous reactions by the body to neutralize these toxic glucose metabolites. Examples of endogenous protective factors are anti-inflammatory and anti-oxidant proteins, which can neutralize the toxic metabolic products of hyperglycemia[21–25]. The origin for postulating the potential existence of endogenous protective factors evolved from our study, the Joslin Medalist Study in people with extreme duration of T1DM. This cross-sectional and longitudinal study characterized

more than 1,000 individuals with 50 years or longer duration of T1DM[26, 27]. In this cohort, only 12% of the Medalists exhibited significant DN with estimated glomerular filtration rate (GFR) $<45\text{mL/min/1.73m}^2$ and 35% exhibited no significant diabetic retinopathy (DR), DN or neuropathy[28]. In addition, the presence and absence of DN or DR did not correlate with history of glycemic control, strongly implying that endogenous protective factors may exist in the Medalists to neutralize the toxicity of hyperglycemia. In the following, we will summarize briefly glucose metabolism in the renal tubular and glomerular tissues and the various pathologies associated with DN. Then, we will describe our recent studies using Medalists and their kidneys to characterize the potential protective mechanisms which may be actuated to neutralize the toxic effects of hyperglycemia.

Various pathological changes in DN

Hemodynamic changes with hyperfiltration are the earliest renal manifestation of diabetes and hyperglycemia[29] (Fig. 2). Pathological lesions of DN involves multiple phases from the initial findings of nephromegaly to the increased thickness of glomerular basement membrane (GBM), and mesangial expansion, hallmarks of DN[30, 31]. Extracellular matrix expansion is a commonly observed histopathological lesion in DN, which ultimately leads to glomerular fibrosis. Classic Kimmelstein-Wilson nodules are only observed at later stages of DN. Tubulointerstitial fibrosis, which is critical to the loss of GFR, is also present in DN in parallel with glomerular pathologies, including accumulation of interstitial myofibroblasts, collagen and inflammatory cells. At the cellular level, glomerular mesangial cells exhibit hypertrophy initially and may even proliferate in parallel with GBM thickening[32, 33]. Podocytes apoptosis results in the loss of the integrity of the glomerular filtration barrier which is comprised of the fenestrated endothelial cell, GBM, along with podocytes. The accumulation of extracellular matrix proteins in the glomeruli and tubules leads to the loss of filtration surface and communications between the glomeruli and the proximal tubules causing declines in GFR and renal function [34, 35].

Glucose metabolism in DN

Glucose metabolism in the kidney is very complex since renal functions include gluconeogenesis, glucose reabsorption, and utilization. During the fasting state, 20–25% of the glucose released into the circulation is derived from the kidney through gluconeogenesis[36]. Approximately 180 grams of glucose are reabsorbed in the renal tubules through sodium glucose transporters, which can increase in diabetes. Glucose and free fatty acids are the major sources of fuel for renal metabolism[37]. Other than renal tubules, most of the renal cells especially glomerular cells utilize GLUT1 for glucose uptake, although a small number of GLUT4 may exist[38]. Clearly, hyperglycemia is the major causal factor for diabetes-specific pathologies in the glomeruli and proximal tubules[39]. In addition, kidney diseases can also occur in obese people without hyperglycemia although the prevalence is significantly lower than those with consistent hyperglycemia and diabetes [40]. Thus, our review will focus mainly on glucose related changes in the glomeruli with brief description of proximal tubules. The major source of metabolism in the tubules is probably free fatty acids, as opposed to glucose in the glomeruli. Metabolically, the renal tubules are much more active than the glomeruli. Adding

to the complexity, insulin has multiple and differential actions in the glomerular cells compared to the proximal tubular cells. For the glomerular cells, insulin is known to regulate the expressions of eNOS and VEGF in glomerular endothelial cells and podocytes[41–43], respectively. In the proximal tubular cells, insulin can regulate sodium transport[44]. In the insulin resistance state, glomerular cells appeared to exhibit selective insulin resistance similar to other vasculature and non-renal tissues in the body with loss of signaling via IRS/PI3-kinase/Akt in a rat model[45]. However, multiple studies have shown that the actions of insulin in proximal tubular cells regarding sodium transport are maintained even in insulin resistance states leading to the well noted clinical finding of sodium retention and edema after the initiation of insulin treatment in diabetic patients[46],[47]. This complexity of differential glucose uptake and metabolism in different renal cells is likely one of the reasons for the various pathologies observed in renal glomeruli and proximal tubules in diabetes[46, 48–51]. One possible explanation for the differences in cellular reaction to hyperglycemia could be due to the physiological exposure of renal proximal tubules to persistent elevation of intracellular glucose due to its functions of uptake and reabsorption of glucose from glomerular filtrates. In contrast, glomerular cells are only exposed to plasma glucose levels, which are not elevated in non-diabetic states[52].

Effects of elevated intracellular glucose on glomerular cells

As described above, it is very likely that elevation of glucose in the intracellular compartment will be processed differently in the glomerular cells compared to proximal tubular cells (PTC) due to the greater demand for fuel in the latter and the additional function of the PTC to transport glucose from intra-tubular space into the cells using SGLT transporters before metabolize or transport them into the vascular space. Thus, PTC may have additional adaptive pathways to process the extra intracellular free glucose load. Next, we will discuss the potential effects of elevating intracellular glucose in the glomerular cells since our new studies have targeted mainly podocytes[48]. Glomerular cells are mainly comprised of endothelial cells, mesangial cells and podocytes. It is likely that glucose metabolism in these cells are different since their responses to hyperglycemia and diabetes are different with increased apoptosis for the podocytes, and possibly, the endothelial cells, but mesangial cells initially exhibit hypertrophy and even proliferation at early stages of DN[42]. These differential responses to hyperglycemia in the glomerular cells could be due to the differences in glucose metabolism or the responses of the cells to the glucose metabolites. Since all the glomerular cells mainly express GLUT1 for the transport of glucose from extracellular to intracellular compartments, it is likely that the metabolism of glucose in all three types of cells are increased in diabetes. One potential difference in glucose metabolism in the glomerular cells could be the cellular expression of GLUT1 in response to hyperglycemia. For example, elevation in external glucose does not change the protein level of GLUT1 in endothelial cells, but downregulates GLUT1 expression in vascular smooth muscle cells[53]. Thus, it is possible that mesangial cells and podocytes could have differential changes in GLUT1 expression when chronically exposed to hyperglycemia.

Once glucose enters the cells, it is metabolized via glycolysis with phosphorylation by hexokinase, which has K_m (Michaelis constant) less than 1 mM[54]. However, elevation of

intracellular glucose can also increase metabolism of glucose via aldose reductase/sorbitol dehydrogenase pathway, which exhibits K_m more than 5 mM[55]. Multiple studies have shown that incubation of these glomerular cells with high glucose culture media comparable to hyperglycemia in diabetes will increase flux via aldose reductase pathway, elevate level of diacylglycerol (DAG), formation of methylglyoxal, and increase production of ROS in the mitochondria[16, 56–58]. These studies have suggested that elevation of sorbitol or activity of aldose reductase may lead to various vascular complications, including DN, due to the changes in nicotinamide adenine dinucleotide (NAD)[12]. Increases in methylglyoxal levels can cause non-enzymatic glycation of proteins in either intracellular or extracellular spaces, which induced the dysfunction of glomerular and other vascular cells[59]. Further, increased glucose metabolism will result in excessive ROS production via oxidative phosphorylation in the mitochondria or due to activation of NADPH oxidase (NOX)[16, 60]. In addition to the increased formation of potential toxic metabolites, high glucose levels also increases the formation of DAG via glycolysis which subsequently activates PKC and other stress kinases, such as p38 MAPK and further cause signaling dysfunctions and vascular abnormalities, even apoptosis[61]. However, multiple clinical trials using aldose reductase inhibitors, antioxidants, anti-actions of AGE and anti-inflammation have not delivered positive or significant clinical outcomes. The lack of efficacy using inhibitors targeting these pathways suggests that a single pathway as described above may not be responsible for the development DN. It has also been postulated that that mitochondrial ROS alone is possible responsible for the abnormalities observed in many of these pathways. Thus, targeting mitochondrial dysfunction and production of ROS could be potential treatments for the development of DN or other vascular complications in diabetes. However, a recent study in a mouse model of T1DM has suggested that ROS production may be increased or decreased depending on the stages of DN[62]. Mitochondrial defects in both renal tubular and glomeruli have been described in diabetes, especially with oxidative stress and fibrosis. In renal tubular epithelial cells defects in fatty acid oxidation, a critical source of ATP, have been reported [49]. For the glomeruli, mitochondrial defects also have been reported for the podocytes and the endothelial cells[63].

Previously, mitochondrial defects were postulated as results of increased flux via glycolysis or epigenetic changes to altering several proteins, such as PGC-1 α and oxidative phosphorylation enzymes, resulting in elevated oxidant production. Studies by Long et al[64] suggested that reduction of long non-coding RNA Tug 1 in diabetes could be partially responsible for decreasing PGC-1 α in the podocytes leading to mitochondrial defects and elevation of ROS. However, the mechanism by which diabetes or hyperglycemia is causing the reduction of Tug 1 and PGC-1 α was not described[64]. Due to the lack of clear mechanisms to explain the pathways that hyperglycemia is causing DN, we postulated that protective mechanisms may exist in the glomeruli to allow the Medalists to survive with T1DM for more than 50 years without DN. In the following, we will describe the steps which were used to identify potential protective mechanisms, which could neutralize the adverse effects of hyperglycemia on glomerular cells and delay DN.

Characterization of metabolic profile in glomerular cells that protects from DN

We embarked on a new perspective to clarify the development of DN by characterizing factors which could protect diabetic patients from DN, even with prolonged duration of disease and exposure to hyperglycemia. As described above in the Joslin Medalists, those with T1DM for 50 years or longer, only 12% exhibited significant DN ($\text{GFR} < 45 \text{ mL/min/1.75 m}^2$) [28]. To identify potential protective mechanisms, glomeruli were isolated from postmortem kidneys in those patients with or without DN with comparable glycemic control and differences in protein expressions were determined by proteomic analysis using mass spectrometry [48]. The results showed clear differences in protein profiles between glomeruli with and without significant DN, especially involving proteins mediating glucose or hexose metabolism. We found that multiple enzymes in the pathways of glycolysis, aldose reductase, methylglyoxal and tricarboxylic acid (TCA) cycle were elevated in the protected kidneys. In parallel, we also observed that glucose metabolites, which are the substrate of these enzymes changed inversely with the enzymes involved (Fig. 3). The findings that enzymes in the glucose metabolism pathways were elevated suggested that lowering intracellular free glucose in glomerular cells is important for the maintenance of cell function and survival. These results suggest that enhanced metabolism of intracellular glucose appears to protect glomerular functions probably preventing or reversing mitochondrial dysfunction. In addition, elevation of glyoxalase I and glyoxalase II indicated that reduction of methylglyoxal is also important to preserve glomerular or podocyte function and survival (Fig. 3). One clear example that increased metabolism of intracellular free glucose and some of its metabolites are important in protecting podocyte or glomerular cells is the finding of increased expression of aldose reductase (AR) and sorbitol dehydrogenase (SODH) in the protected kidneys compared to those with DN indicating that increased flux through the sorbitol pathway is also important to preserve glomerular function. This hypothesis is in contrast with the previous long-held theory that increased flux through the sorbitol pathway could be protecting the glomerular cells by increasing aldose reductase activity or flux causing DN and other vascular diseases, either by increased osmotic pressure from elevated levels of sorbitol or alteration of NAD/NADH ratio via the increased flux [65]. However, by analyzing the protected kidneys, our study suggests the opposite, that increased flux via the sorbitol pathway actually maintains renal function and prevents from DN. These new results derived from the Medalist study potentially provide an explanation for the lack of efficacy of AR inhibitors in clinical trials for DN.

The idea that increased flux through the various glucose metabolic pathways to maintain intracellular glucose levels and prevent glomerular or podocyte dysfunction was further confirmed by our studies in the cultured podocytes and diabetic rodents. To test the hypothesis that increased flux in glycolysis could be protective, we elected to activate pyruvate kinase M2, which along with PKM1, are the major enzymes converting phosphoenolpyruvate to pyruvate and could potentially regulate glycolytic flux and TCA/mitochondrial pathways [66]. Using a series of strategies either to activate PKM2 by small molecule activators (TEPP-46), or to delete PKM1 or PKM2, we observed that PKM2 configuration shifts from monomeric or dimeric to tetrameric isoforms can induce glucose

flux via glycolysis and decrease glucose metabolisms via sorbitol, methylglyoxal, and DAG synthesis pathways, especially when podocytes are exposed to elevated glucose levels to mimic hyperglycemic conditions (Fig. 4). Further, inhibition of PKM2 enhanced the toxic effect of elevated glucose levels with parallel accumulation of sorbitol, methylglyoxal and DAG/PKC activations. Functionally, activation of PKM2 improved mitochondrial dysfunction, its membrane potential (MMP), and DNA content, decreased ROS production, and increased mitochondrial biogenesis gene expressions such as PGC1 α . Pathologically, expression levels of extracellular matrix proteins, inflammatory cytokines, and fibrotic growth factors, such as TGF β and CTGF, were decreased or increased respectively with activation or deletion of PKM2 in podocytes or *in vivo* in diabetic mice. Further, intervention studies in diabetic mice showed that PKM2 activation, by TEPP-46, even after 3 months of diabetes, can stop or reverse many of the abnormal glomerular changes with an additional 3 months of treatments. This is very exciting since it suggests that increase in glycolysis flux can reverse metabolic memory induced by hyperglycemia or diabetes which is known to be difficult to reverse even after multiple years of good glycemic control for DN, and other vascular complications[5, 67, 68] [69]. The mechanistic explanation of metabolic memory caused by hyperglycemia is unclear. Several hypotheses have been proposed including epigenetic modifications via acetylation or methylation of histones, which could potentially modulate the expressions of genes either by elevated level of S-adenosylmethionin (SAM), α -ketoglutarate (α -KG) or Fe²⁺/Fe³⁺ [70]. Another potential explanation for metabolic memory could be due to the slow turnover of extracellular matrix proteins, such as those in the basement membrane, which can cause abnormalities in vascular cells[71, 72]. Thus, the post-translational modifications and turnover of extracellular matrix proteins are likely major causes for the manifestation of metabolic memory. Our study suggests that improvement in glycolysis and its metabolites in glomerular cells, such as podocytes, may induce mitochondrial biogenesis, which can reverse the epigenetic changes or alternatively, accelerate extracellular matrix turnover and remodeling in order to rapidly reverse metabolic memory. Our data strongly suggests that lowering intracellular free glucose by increasing metabolic flux of glucose in podocytes and other glomerular cells can potentially promote the regeneration of mitochondria which will subsequently improve glomerular function, survival of glomerular cells and delay the progression of DN in the presence of hyperglycemia (Fig. 4). It is also interesting to note the differential effect of the activation of PKM2 and PKM1, since overexpression of PKM1 was not able to reverse the adverse actions of hyperglycemia compared to PKM2. These differential effects of PKM1 and PKM2 suggest that these two proteins derived from the same gene exhibit significant differential effects in podocytes[48]. Future studies will be needed to understand their differences in regulatory function of glucose metabolism, possibly by the differences in their subcellular location or interactions with different sets of intracellular proteins. In addition, further studies will be needed to identify mechanisms for the up-regulation of so many glycolytic enzymes in the protected renal glomeruli.

Our studies using information derived from renal glomeruli of diabetic patients who were protected from nephropathy, even after extreme exposure to diabetes and hyperglycemia have suggested that maintenance or elevation of metabolic enzymes of glucose appeared to be protective. The elevations of these enzymes of glucose metabolism appear to prevent the

elevation of intracellular free glucose in the podocytes and decrease levels of methylglyoxal, ROS, and lipid precursors, as well as improved mitochondrial function. The orchestration of this complex metabolic system is to increase glucose flux in the podocytes, prevent or reverse podocyte dysfunction, improve their survival and renal function in diabetic patients. Further intervention studies will be needed in order to test this hypothesis that activation of PKM2 at the molecular level can activate glycolysis, especially in the renal glomeruli and stop, or even reverse glomerular dysfunction in diabetic patients.

In summary, we propose that hyperglycemia causes DN by multiple changes including alterations of hemodynamics such as hyperfiltration and increased glomerular pressure. In addition, elevation of intracellular free glucose in the glomerular and tubular cells can affect their function and survival. Our study suggests that both metabolic and hemodynamic abnormalities are required for the progression of DN. Thus, effective treatments to prevent or even reverse the pathologies of DN in the presence of hyperglycemia will not only need to normalize the hemodynamic changes using agents, such inhibitors of renin-angiotensin system, but also need to protect against intracellular hyperglycemia by increasing glycolytic flux, possibly by activating PKM2. This concept of hyperglycemia causes DN by altering both hemodynamics, and metabolism to cause dysfunction of renal vascular, glomerular, and tubular cells in parallel is likely since therapeutics that only affect hemodynamics or metabolism in diabetic rodent models or clinical studies have not met with success to stop or reverse DN.

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Fig. 1.
Concept of hyperglycemia induced toxic induced pathways and neutralizing responses.

Fig. 2.
Progressive steps of hyperglycemia/diabetes induced renal dysfunction and nephropathy.

Fig. 3. Proteomic and plasma metabolomics signatures of Medalists without DN. Glomerular glycolytic enzymes (red) were up-regulated with corresponding decreased plasma metabolites (blue) in the Medalist without DN.

Fig. 4. Schematic diagram of metabolism changes in podocytes induced by hyperglycemia and its normalization by PKM2 activation. In diabetes or hyperglycemia, PKM2 is oxidized or phosphorylated at Y¹⁰⁵ site which configures PKM2 to dimer or monomer isoforms, resulting in decreased PK activity. Lower PK activity causes the accumulation of toxic glucose metabolites/molecules in the pathways of sorbitol/AR, MG and PKC. Lower PK activity results in decreased glucose flux via TCA cycle and causes mitochondrial dysfunctions and overproduction of ROS. These adverse events will subsequently cause podocytes apoptosis and promote the development of DN. Therapeutic treatment using small

molecule of PKM2 activator can potentially reverse the above events and slow or prevent DN progression.

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