Title

Sodium-glucose cotransporter-2 inhibitors for diabetic kidney disease: Targeting Warburg effects in proximal tubular cells

Author(s)

Miwa Morita, Keizo Kanasaki

Journal

Diabetes & metabolism, 46(5):353-361.

Published

2020 Oct

URL

https://doi.org/10.1016/j.diabet.2020.06.005

Sodium glucose cotransporter 2 inhibitors on diabetic kidney disease: targeting Warburg effects in proximal tubular cells

Miwa Morita, Keizo Kanasaki

1) *Department of Internal Medicine 1, Faculty of Medicine, Shimane University, 89-1 Enya-cho, Izumo 693-8501, Japan*
2) *Division of Anticipatory Molecular Food Science and Technology, Medical Research Institute, Kanazawa Medical University, Uchinada, Ishikawa, Japan 920-0293*

Address correspondence to:

Keizo Kanasaki, MD, PhD
E-mail: kkanasak@med.shimane-u.ac.jp
Department of Internal Medicine 1, Faculty of Medicine, Shimane University, 89-1 Enya-cho, Izumo 693-8501, Japan

Abstract

Inhibitors of sodium glucose cotransporter 2 (SGLT2) undoubtedly shift the paradigm in diabetic medicine/research, especially for diabetic kidney disease (DKD). The pharmacological action of SGLT2 inhibitors is as simple as releasing glucose into urine; however, how SGLT2 inhibitors contribute to the health of diabetic people has not been completely elucidated. Here, we provide a novel insight of the action of SGLT2 inhibitors by introducing a neglected fuel-burning system in proximal tubular cells, "glycolysis." Exploring the details of the molecular mechanisms and clinical biomarkers of the organ protection conferred by SGLT2 inhibitors is required for the preparation for the next stage of diabetic medicine in the clinic, a "post-SGLT2 inhibitor era."

Key words: SGLT2, glycolysis, sirt3, PKM2, hypoxia-inducible factor

Introduction

Diabetes is a global health threat, with 425 million people affected in 2017, that is expected to increase to 629 million by 2045. Diabetic kidney disease (DKD) is the major cause of end-stage renal disease worldwide, and no specific therapy is available to halt its progression. In addition to therapies appropriate for controlling major risk factors, including those directed to blood glucose management and blood pressure control, the most relevant therapy to combat DKD is a renin-angiotensin system (RAS) inhibitor; it is apparent that a significant residual risk of DKD remains. Substantial efforts have been made to discover alternative therapies to halt DKD progression, but none makes a significant contribution to stopping DKD expansion yet. Therefore, significant unmet needs are driving diabetes researchers to discover alternative strategies to mitigate DKD progression.

In this regard, we have been living in the dark for the past 20 years, with insignificant progress from the clinical utility of the RAS inhibitors against DKD. However, in the past 5 years, we have witnessed an astonishing outcome in DKD research, a diabetic drug that releases glucose into urine: the sodium glucose cotransporter 2 (SGLT2) inhibitor.[1]

Glucose reabsorption by kidney and the SGLT2 inhibitors

Over the past 20 years, considerable data have accumulated indicating that the human kidney is involved in the regulation of glucose via gluconeogenesis, taking up glucose from the circulation, and reabsorbing glucose from urine. SGLT2 is expressed on the S1 and S2 segments of the kidney proximal tubule and plays a fundamental role in the reabsorption of urine glucose that has been filtered in the glomerulus.[2] Approximately 90-95% of filtered urine glucose is reabsorbed through SGLT2. This essential biological function of SGLT2 in glucose reabsorption and the lack of significant health problems in patients with renal glycosuria, a population with mutations in the *SGLT2* gene, have led scientists to develop an SGLT2 inhibitor as an antidiabetic drug that releases excess glucose into urine (FIG 1).[2]

Initiating from the discovery of potential renal protection by SGLT2 inhibitors in the EMPA-REG trial,[3,4] later large clinical trials, such as CANVAS [5] and DECLARE TIMI58,[6] confirmed the

renal benefits of SGLT2 inhibitors in high-risk diabetic populations[7] (Table 1). The CREDENCE trial[8] recruited only patients with advanced DKD and found that canagliflozin significantly reduced the primary outcome and kidney-specific outcomes (Table 1). These cardiovascular outcome trials of SGLT2 inhibitors led to groundbreaking clinical practices and basic scientific research on diabetic medicine.

Aerobic glycolysis and sirtuin 3 suppression

Glucose is a macronutrient that can be utilized for diverse biological purposes, such as ATP production and biosynthesis, via the production of macromolecules such as DNA, RNA, proteins, and lipids.[9] The first step of glucose metabolism in the cells, glycolysis, is essential for cell survival in most cell types.[9]

This cellular dependence on glycolysis is much more significant in cells with high energy demands, such as cancer cells.[10] In cancer cells, glycolysis has been observed even under physiologically normal oxygen conditions, and the process by which glucose is fermented to synthesize lactate is called aerobic glycolysis.[10] Later, mitochondrial dysfunction was described as a fundamental event that causes a metabolic shift to aerobic glycolysis. Warburg, who discovered specific glycolysis in cancer cells, hypothesized that mitochondrial dysfunction is essential for aberrant glycolysis in cancer cells, and later, Racker named this aberrant glycolysis in cancer cells as the Warburg effect.[10]

In cancer cells, aberrant glycolysis with dysfunctional mitochondria is associated with the suppression of sirtuin 3 (SIRT3), the major mitochondrial deacetylase that targets several diverse enzymes involved in central metabolism resulting in the activation of many oxidative pathways.[11] SIRT3 functions as a tumor suppressor by maintaining genomic stability[12] and preventing the development of organ fibrosis by regulating transforming growth factor (TGF)-β/smad signaling.[13,14] We have shown that diabetic mice with kidney fibrosis showed significant suppression of SIRT3 and associated aberrant glycolysis. SIRT3 has been shown to inhibit the angiotensin II-stimulated epithelial-mesenchymal transition in renal tubules, which is associated with the suppression of oxidative stress and defects in mitochondria.[15] SIRT3 deficiency augmented cisplatin-induced

nephrotoxicity through the enhancement of renal tubular cell apoptosis and tissue inflammation[16] and aggravated palmitate-induced ROS production in proximal tubular cells and tissue inflammation.[17] SIRT3 deficiency was coupled with the suppression of PGC1α, a master regulator of renal oxidative metabolism in epithelial cells.[18] Smad3, the specific intracellular transcriptional factor for fibrogenic cytokine transforming growth factor β, directly interacts with the regulatory region of the PGC1α gene and inhibits mitochondrial biosynthesis and metabolism.[19] The suppression of PGC1α is associated with SIRT3 suppression in the damaged organs and mitochondrial defects.[20,21] Interestingly, the inhibition of aberrant glycolysis rescued PGC1α levels, thus normalizing SIRT3 levels in diabetic kidneys, suggesting that the PGC1α-SIRT3 axis contributes to homeostasis in mitochondrial metabolism and glycolysis.

Proximal tubular cell glycolysis and fibrogenic program

The proximal tubules reabsorb glomerulus-filtered glucose from urine and can also synthesize glucose. In proximal tubular cells, perhaps in normal status, reabsorbed glucose from urine is not utilized for energy requirements, and most of the energy is generated by the oxidation of free fatty acids.[22] These suppositions were based on the analysis of healthy tubular cells; in cells undergoing the transformation process, the metabolic program is changed.[23,24] Therefore, the utilization and selection of energy sources in these damaged cells is a matter of debate. Alterations to the metabolic program could lead to myofibroblast/precursor accumulation and facilitate fibroblast activation, survival and proliferation.[25,26] Under reduced ambient oxygen conditions (e.g. high altitude) diabetic kidney may not adjust oxygen consumption to meet the oxygen supply and thus present with higher lactate levels and lower redox potential, a shift similar to Warburg metabolism in cancer cells.[27] Glycolysis induction is linked to mitochondrial defects in atrophic tubular cells after acute ischemic kidney injury (AKI) in rats.[28] In the AKI models, several alterations to metabolic programs were reported, such as increased lactate release into the interstitium[29] and elevated pyruvate kinase in kidney homogenates[30]. Diminished fatty acid oxidation after folic acid nephropathy[31] and increased glycolysis after mercuric

chloride-induced AKI, have been reported[32]. Altered levels of methylglyoxal, a byproduct of glycolysis, had a negative association with changed eGFRs, as observed in individuals with type 2 diabetes mellitus over 6 years [33].

Kidney tubules are essential for homeostasis in fluid, electrolytes, and acid-base balance. The GFR is approximately 180 L per day; the average urine output is only approximately 1.5 L per day, and estimates indicate that tubular cells are critical for the reabsorption of 178.5 L per day[34,35] Additionally, tubular cells play roles as endocrine sources. Therefore, it is reasonable to assume that the baseline energy demand of kidney tubular cells is high. However, damaged proximal kidney tubular cells display defects in both fatty acid oxidation and mitochondria.[31,36] In proximal tubular cell lines, defects in fatty acid oxidation have been linked to the suppression of epithelial markers and the induction of mesenchymal markers, such as αSMA, vimentin, intermediate filament, and collagens (epithelial to mesenchymal transition: EMT), suggesting that metabolic reprogramming is associated with EMT induction.[37] In parallel with fatty acid oxidation defects, glycolysis is induced.[37] In kidney tubular cells, the induction of glycolysis serves as a substitutional energy source to compensate for defective fatty acid oxidation; indeed, glycolysis can also participate in this fibrotic mechanism by supplying the metabolic demands of myofibroblasts undergoing transformation.[37,38] Diacylglycerol, a byproduct of the glycolysis pathway, is a key molecule that activates protein kinase C, which is involved in the development of diabetic kidney disease pathogenesis.[39]

Fibrotic mouse model strain displays induction of aberrant glycolysis

The magnitudes of diabetes-associated kidney fibrosis are different among the strain in either type 1[40] or type 2 [41] diabetic mice model. We have utilized STZ-induced CD-1 mice as kidney fibrosis models in diabetes in several papers.[37,42-46] However, until recently, we did not know the major molecular mechanisms responsible for the differences in kidney fibrosis levels between CD-1 mice and other mice with diabetes. We have shown that the fibrotic kidneys of diabetic CD-1 mice displayed

suppressed SIRT3 levels.[37] SIRT3 suppression in the kidneys of CD-1 diabetic mice was linked to TGF-β/smad signaling induction and HIF-1α accumulation with pyruvate kinase muscle isozyme (PKM)2 dimer formation; these alterations subsequently led to aberrant glycolysis with abnormal mesenchymal transformation.[37] Inhibition of aberrant glycolysis by small molecules [2-deoxyglucose (DG) and dichloroacetates (DCA)] resulted in the suppression of fibrogenic programming and restoration of SIRT3 levels.[37] Similar aberrant glycolysis was found in KK/Ta-Ins2Akita mice, constituting a model of progressive diabetic kidney disease.[37] In vitro, in the HK2 tubular cell line, TGF-β1-induced aberrant glycolysis and the EMT were inhibited by DCA. In contrast, SIRT3 knockdown induced aberrant glycolysis and EMT; DCA failed to inhibit EMT in SIRT3-knockdown HK2 cells. In vivo suppression of SIRT3 by siRNA augmented fibrogenic programs in CD-1 mice. These data suggest that SIRT3 is essential for both the inhibition of aberrant glycolysis and the disruption of a balanced epithelial cell phenotype, affecting antifibrogenic homeostasis (FIG 2).[37]

The higher levels of glycolytic protein were associated with the induction of the PKM2 dimer, the inactive form of this kinase, and suppression of the PKM2 tetramer. In contrast to the cytoplasm-localized PKM2 tetramer, the PKM2 dimer accumulates in the nucleus and binds to several transcriptional factors, including HIF-1α[47]. The PKM2 dimer has been shown to enhance, perhaps somewhat detrimentally, HIF-1α transcriptional activity and therefore induces the transcription of glycolysis-promoting enzymes, subsequently causing glycolytic intermediates to accumulate as metabolites at higher levels than the level of the pyruvate kinase necessary for the synthesis of nucleic acids, phospholipids, glycoproteins, and amino acids and O-linked glycosylation, among other functions[47]. Recently, Joslin investigators demonstrated that the restoration of low active form of PKM2 by TEPP-46, the PKM2 activator, inhibited fibronectin, type I collagen α3, and TGF-β1 expression levels in the tubular lesion; in contrast, the effects of TEPP-46 were minimal in the glomeruli of experimental animals.[48] These data demonstrated that aberrant glycolysis, particularly PKM2 dimer formation, may be a potential therapeutic target to halt kidney damage, especially in diabetic patients.[48]

SGLT2 inhibitor: focus on the pathogenic significance of reabsorbed urine glucose and aberrant glycolysis

As described in the previous paragraph, aberrant glycolysis in proximal tubules is a provocative target in DKD therapy. However, glycolysis in kidney proximal tubules has long been ignored and is rarely analyzed. Moreover, the pathological significance of urine glucose has never had much appeal. One of the reasons why proximal tubular cell glycolysis and urine glucose have been ignored as pathomechanisms of kidney injury may be based on the belief that reabsorbed glucose from urine is directly transported out from the basolateral side. In the 1960s and 1970s, it would have been common sense to suggest "no or minimal glycolysis happens in proximal tubules."[49,50] However, recent reports have demonstrated that nonnegligible glycolysis is undertaken in proximal tubules, even those in a normal state, as revealed by multiphoton microscopy analysis,[51] and even several stress conditions have been associated with the augmentation of glycolysis in damaged proximal tubules.[52,53] To support the implication for a pathogenic role of glycolysis, the inhibition of glycolysis suppressed kidney fibrosis in a unilateral ureteral obstruction model.[54] mTOR activation is linked to glycolysis.[55] Both mTOR activation and aberrant glycolysis have been shown to contribute to the pathogenesis of polycystic kidney disease.[56-61] Indeed, either the mTOR inhibitor-[62] or β-hydroxybutyrate-, a potent inhibitor of glycolysis,[63] mediated mTOR inhibition,[58] halted the progression of polycystic kidney disease.[59] Interestingly, tubule-derived lactate has shown to be required for fibroblast activation in an acute kidney injury model.[64]

The proximal tubule of the diabetic kidney carries an immense amount of glucose reabsorbed from urine via SGLT2; however, the pathological relevance of urine-derived glucose has not yet been established. Even worse, SGLT2 levels have shown to be increased in diabetic kidneys.[65,66] Therefore, proximal tubular cells cannot adjust or otherwise appropriately reduce glucose transport rates to prevent excessive glucose accumulation under high-glucose conditions.[65] SGLT2-mediated glucose uptake from urine was coupled with the export of glucose from proximal tubular cells from the basolateral side through GLUT2 via a glucose concentration-dependent gradient. The

exact ratio of glucose uptake by SGLT2 to glucose export by GLUT2 in vivo and in humans has not yet been elucidated. In theory, if the glucose concentration is higher on the basolateral side, such as under diabetic conditions, to establish the necessary GLUT2 gradient, intracellular glucose levels in proximal tubular cells must be higher (FIG 3). Additionally, no one knows whether GLUT2 function is appropriate in diabetic conditions and/or kidney damage. Regarding GLUT2 in the proximal tubule, GLUT2 deficiency in humans is associated with Fanconi nephropathy, a disease manifesting proximal tubular cell dysfunction and damage.[67-70] Therefore, urine-derived glucose transport defects on the basolateral side of the proximal tubular cells could lead to kidney damage (FIGs 2 and 3). Diabetic conditions are associated with both high glucose on the basolateral side and SGLT-mediated uptake of high urine glucose levels filtered in glomeruli [65,66]. The mechanism by which glucose levels alter or cause fluctuations in urine glucose transport to the basolateral extracellular space has not been sufficiently analyzed.

The reason that SGLT2 inhibition can inhibit aberrant glycolysis has not yet been clearly revealed. From our previous report on the kidney fibrosis phenotype in diabetic mice, the glycolysis phenotype was not simply explained by the blood glucose levels [46]. Our previous report demonstrated that the significant difference between fibrotic and nonfibrotic mouse strains could be based on the levels of sirtuins, such as SIRT1 and SIRT3[37], NAD^+-dependent deacetylases.[71] Even though major interest in diseases of aging area focusing on this deacetylase, the regulation of SIRT3 levels has not yet been clearly shown.[71] Regard with this, alterations in NAD^+ could occur in diabetic mice, even though we did not measure that in the particular fibrotic kidney of CD-1 diabetic mice. SIRT1 is mainly localized in the nucleus, but SIRT3 is localized in mitochondria.[71] NAD^+ levels may affect the levels of both sirtuins in the kidney; the difference cannot be simply explained. Other than NAD^+, ERRα, and PGC-1α have been shown to contribute the regulation of SIRT3[72] and the details on the regulation of these SIRT3 regulators associated with SGLT2 have not yet been elucidated.

Restoration of SIRT3 levels by SGLT2 inhibition is key to understanding antiglycolytic systems and antifibrotic pathway activation. We have previously shown that SGLT2 inhibition leads to suppressed aberrant tubular glycolysis,[46] and others showed that it induces gluconeogenesis,[73] indicating a logical connection between alterations to tubular glucose metabolism and SGLT2 inhibition. We have also shown that SGLT2 inhibition suppresses glycolysis flux to a level similar to that of nondiabetic CD-1 mice and is associated with the suppressed accumulation of HIF-1α, P-STAT3 and PKM2 dimer formation,[46] the typical alteration in aberrant glycolysis.[74,75] Whether glycolysis inhibition and/or gluconeogenesis induction in tubules can be attributed to the amelioration of kidney injury by SGLT2 inhibition is unknown; we have shown that the glycolysis inhibitor 2-DG suppresses EMT and fibrosis in the kidney.[37] The accumulation of HIF-1α and P-STAT3 has been linked to the EMT in kidney tubules.[76,77]

PKM2 forms a complex with HIF-1α; this complex can recruit the p300 acetyltransferase, which enhances HIF target gene transactivation.[78] Tumor cells are often in hypoxic environment and such hypoxia induces the interaction of JMJD5 (Jumonji C domain-containing dioxygenase) with PKM2.[79] JMJD5-PKM2 interaction subsequently accelerates the PKM2 nuclear translocation to facilitate HIF1-mediated glycolytic enzyme transcriptional activation to support cancer cell survival.[79] In addition, nuclear PKM2 is likely in dimer form and directly phosphorylates Y705 of STAT3, even when neither the Jak2 nor c-Src pathways are activated, and PKM2-phosphorylated STAT3 activates the transcription of a number of STAT3-targeted genes.[80] In addition to its effect on HIF-1α and STAT3, PKM2 acts as a kinase to stimulate numerous transcriptional factors that are relevant to glycolysis and fibrosis.[81]

In addition to PKM2, fibrogenic cytokines such as transforming growth factor (TGF)-β and interleukin (IL)-6 appear to be glycolytic stimuli[82,83] and activate STAT3.[84,85] STAT3, a downstream transcriptional factor activated by mTOR signaling, induces glycolysis by targeting hexokinase 2 in hepatocellular carcinoma cells.[86] In CD8 T cells, STAT3 activation is also linked to fatty acid oxidation.[87] As described above, in the diabetic kidney, fatty acid oxidation is significantly

diminished, suggesting that STAT3 activation can trigger compensatory mechanisms that restore fatty acid oxidation in tubular cells. The constitutively active form of STAT3 (Y-P STAT3) promotes aerobic glycolysis and suppression of mitochondrial activity, partly by acting through the transcriptional activation of HIF-1α targets.[88] Regardless of the mechanism, the functional interaction between STAT3 and HIF-1α is relevant to the specific binding of STAT3 to the promoter of HIF-1α target genes, forming active HIF-1α/RNA Polymerase II transcriptional complexes.[89] Thus, SGLT2 inhibition can suppress aberrant glycolysis via the restoration of SIRT3 and the subsequent inhibition of STAT3, HIF-1α and PKM2 cross talk (FIG2).[80,90]

There has been no clear demonstration of the link between the inhibition of aberrant glycolysis and SGLT-2 inhibitor use in human DKD. Plasma lactate levels has been shown to systematically increase in people with diabetes, and SGLT2 inhibition has been shown to suppress lactate levels.[91] However, plasma lactate at this level cannot be simply attributed to the kidneys; therefore, reliable biomarkers to detect kidney glycolysis are necessary but have not yet been established.

SGLT2 inhibition may benefit neighboring cells

The EMT in the kidney has been the focus of debate, and some communities doubt that kidney epithelial cells undergo EMT.[92] We support these dynamic discussion and suggest that the EMT may be incomplete; that is, the complete conversion of epithelial cells into fibroblast may be a rare or nonevent. However, others confirmed, at minimum, an extant epithelial to mesenchymal transition phase.[93,94] Here, we would like to emphasize that this transition program in epithelial cells may influence neighboring cells via the induction of transition programs that lead to the acquisition of a mesenchymal phenotype.

In general, it is logical to assume that SGLT2 inhibitors must primarily display their pharmacological effects on kidney proximal tubular cells. In our analysis,[46] SGLT2 inhibition restored the epithelial phenotype in proximal tubular cells and inhibited endothelial-mesenchymal transition (EndMT) in peritubular endothelial cells (PECs) as well. The conditioned medium experiment using SGLT2-knockdown HK2 tubular cells also demonstrated that epithelial SGLT2 contributes to the production of fibrogenic paracrine factors.[46] These data suggest that SGLT2-mediated glucose entry into tubular cells plays, at least, a pathogenic role in neighboring cells via paracrine factors; diabetic kidney tubular cells, which absorb massive amounts of glucose, are damaged, and these cells acquire matrix-producing cell phenotypes, expressing mesenchymal proteins, such as αSMA and FSP1. Not in the diabetic mouse model, in other fast progressive aggressive kidney fibrosis model, pericyte or resident fibroblasts has shown to be major source of myofibroblast or active fibroblast,[95] such phenotypic alteration in pericyte could be also influenced by paracrine factor from epithelial cells. Indeed, erythropoietin, the hematopoietic factor produced in kidney pericytes, has shown to be suppressed in diabetic individuals and is increased by SGLT2 inhibitor administration.[96] This finding suggests that an SGLT2 inhibitor can restore the tubular phenotype from the EMT to normal form, suppress paracrine factors, and subsequently normalize the erythropoietin-producing pericyte phenotype.[46] Kidney fibrosis may progress because of the interactions among any types of kidney cells.[92] Therefore, our data strongly indicates that the restoration of proximal tubular cells from EMT by SGLT2 inhibitor mitigates such paracrine factors-induced fibrogenic interaction among kidney cells (FIG2).

Glomerular injury: how it connects to tubular cell phenotype

As described above, SGLT2 inhibitors have a dominant effect on proximal tubular cells. However, as evidenced by clinical trials,[97] SGLT2 inhibitors suppress urine albumin excretion, and we confirmed a significantly reduced albumin:creatinine ratio (ACR) in diabetic mice, a finding similar to that of

other studies. In addition, in our analysis, empagliflozin treatment mitigated the podocyte phenotype and mesangial expansion in diabetic mice. To explain the glomerular phenotypic alterations, the most sophisticated hypothesis suggests the recovery of the tubule-glomerular feedback mechanism, which is diminished in diabetic kidneys.[98] How the glycolysis phenotype in kidney tubules could functionally influence hemodynamic alterations has not yet been analyzed. There are some evidences that suggest the tubular phenotype may be linked to the glomerular phenotype. In our fibrotic mouse strain analysis, SIRT1 levels were not altered and therefore was not relevant for our model; however, the working hypothesis suggesting the suppression of proximal tubular SIRT1 levels and subsequent podocyte injury via a reduction in tubule-derived nicotinamide, the substrate for podocyte SIRT1, has been proposed.[99] Tubulointerstitial injury is directly connected to kidney parenchymal hypoxia, by which global kidney damage, including glomeruli, can be induced.[100] Interestingly, some papers have shown that SGLT2 is expressed in glomerular mesangial cells[101] and podocytes.[102] Additionally, in early lung cancer cells, the SGLT2-mediated uptake of glucose plays a pathogenic role.[103] The authors believe that, SGLT2 in the tubular cells plays a dominant role in the development of diabetic kidney injury; however, for the reasons outlined above, it is difficult to discriminate whether SGLT2 in proximal tubular cells and/or in other types of cells manifest dominant pathogenic roles. Experiments utilizing the inducible SLGT2 knockdown in specific cell type would be required for further study.

Perspective: looking to a post-SGLT2 inhibitor era

As described above, the renoprotective effects of SGLT2 inhibitors are gradually becoming clear. However, from the translational research point of view, inhibition of hyperfiltration with the restoration of tubuloglomerular feedback mechanisms may be the most relevant theory. A clearer demonstration of the underlying mechanisms based on analyses of previous clinical trials is needed.

To this point, a sub-analysis of the EMPA-REG renal outcome study provided important clues to understand the renoprotective effects of SGLT2 inhibitors. Cherney et al. analyzed EMPA-REG study data on basal urine albumin levels[104] and showed that empagliflozin had distinct renoprotective effects and induced alterations in parameters of renal function. In their analysis,

empagliflozin suppressed urine albumin levels, even in normoalbuminuric diabetic patients, compared to the levels induced by the placebo; after the intervention period (average 2.6 years), the urine albumin levels had increased to the levels in the placebo group after a washout period of 5 weeks.[104] Interestingly, the patients with albuminuria displayed a different pattern: the empagliflozin treatment suppressed urine albumin levels in both the microalbuminuria and macroalbuminuria groups, as expected; however, after the intervention and wash-out period, the empagliflozin-treated group continued to display low urine albumin levels compared to those of the placebo group.[104] The renoprotection by SGLT2 inhibitors[7] taken with the finding of divergence/fluctuation of urine albuminuria caused by empagliflozin suggest that the SGLT2 inhibition by empagliflozin is not limited to pure hemodynamic effects, and especially in patients with advanced-stage diabetic kidney disease, SGLT2 inhibitors may exhibit renoprotective effects by morphological amelioration in the diabetic kidney. However, the characteristics of those who did and did not respond to SGLT2 inhibitors are not reported, at least in the publication base. All exciting raw data should be included in all clinical studies, and we sincerely request these data for use in further analysis when addressing unanswered questions in the future; for example, is suppression of urine albumin important? Is the initial dip of eGFR in response to SGLT2 inhibition important? Also, we eager to endeavor the clinical biomarkers for estimating the glycolytic status in the kidney tubule.

Conclusion

Our analysis, focused on glycolysis in kidney tubules, is solely one aspect of beneficial effect of SGLT2 inhibitors. Indeed, many theories have been proposed following the provocative clinical data of SGLT2 inhibitors on renal outcomes.[7,8] To date, the most rational clinical theory could be the normalization of tubule-glomerular feedback mechanisms and subsequent suppression of glomerular hypertension in the diabetic kidney,[98] which are classical glomerular injury molecular mechanisms. Other theories could be involved but no single theory can explain the whole story of renoprotection by SGLT2 inhibitors (FIG 4). Tubular glycolysis theory,[37,46] on which we focus, can explain, at least in part, how the inhibition of SGLT2 in the renal tubule directly affects the tubule metabolic pathways

associated with the inhibition of the EMT and paracrine factors and thereby the restoration of neighboring cell phenotype as well (FIGs 2-4).

The discovery of SGLT2 inhibitors opens the door for a new paradigm shift away from the past 20 years of stagnant advances to a "post-SGLT2 inhibitor era" in the research of DKD.

Acknowledgments

Authors declare there is no competing interest associated with this review manuscript. The associated study was primarily supported by a grant from the Japan Diabetes Foundation (2016), and partially supported by grants from the Japan Society for the Promotion of Science to KK (19K08738) and the Uehara Memorial Foundation to KK (2019). KK collaborated with Boehringer Ingelheim at both Kanazawa Medical University and Shimane University. Boehringer Ingelheim, Mitsubishi-Tanabe Pharma and Ono Pharmaceutical contributed to establishing the Division of Anticipatory Molecular Food Science and Technology. KK is under a consultancy agreement with Boehringer Ingelheim.

References

Table 1 Cardiovascular and renal outcomes in clinical trials with SGLT2 inhibitors

Figure Legends

Figure 1 Mechanism of action of SGLT2 inhibitors. SGLT2 inhibitors primarily function with the S1 and S2 segments of the proximal tubule of the kidney. Inhibition of SGLT2 results in the enhanced delivery of glucose to the downstream urinary tract and is associated with theoretically higher sodium levels in urine. Higher sodium levels are monitored by the Na-K-2Cl cotransporter (NKCC2) of the macula-densa; driven by activated NKCC2, adenosine is released into glomerular capillaries. Adenosine contracts afferent arterioles and dilates efferent arterioles, subsequently suppressing glomerular hyperfiltration/intraglomerular pressure.

Figure 2 SGLT2-mediated reabsorbed glucose induces aberrant glycolysis. under diabetic conditions, glucose filtered from the glomerulus is increased; therefore, the glucose reabsorbed by SGLT2 in proximal tubular cells is also greatly increased. This accumulated glucose theoretically is transferred to the vascular side by a glucose gradient-dependent transport established by GLUT2; under diabetic conditions, this accumulated glucose in the proximal tubule is expected to be higher and persistent. This accumulated glucose may suppress sirt3 levels. Sirt3 suppression subsequently induces HIF1α accumulation, PKM2 dimer formation, and STAT3 phosphorylation; consequently, aberrant glycolysis is induced. This aberrant glycolysis is utilized for the transformation process or the maintenance of fibrotic programs. Alternatively, glycolysis-induced energy is utilized to meet the metabolic demands of proximal tubular cells.

Figure 3 Difference in the glucose gradient between the proximal tubule and vascular side of the extracellular space in diabetic and nondiabetic kidney disease. Right: in nondiabetic kidney disease patients, due to normal systemic glucose processes, a glucose gradient between the proximal tubule and extracellular space of the vascular side is easily generated. Left top: in diabetic patients, due to the high and unstable levels of glucose processed in the body, to create a glucose gradient

between the proximal tubule and extracellular space of the vascular side, proximal tubular cells require higher levels of intracellular glucose. Left bottom: However, due to unstable systemic processes, glucose levels in the plasma/extracellular space may be higher than those of the intracellular proximal tubular cells; in these cases, the glucose flux direction could be from the extracellular space to proximal tubular cells.

Figure 4 Potential renoprotection by SGLT2 inhibitors. The reno-protective effects of SGLT2 inhibitors can be divided by two essential mechanisms, hemodynamic-dependent or -independent, and each mechanism may be further subdivided into detailed mechanisms. None of the single mechanisms can explain the whole picture of reno-protection induced by SGLT2 inhibitors. At minimum, these interactions play vital roles in the renal benefit conferred by SGLT2 inhibitors.

1 Delanaye, P. & Scheen, A. J. Preventing and treating kidney disease in patients with type 2 diabetes. *Expert Opin Pharmacother* **20**, 277-294, doi:10.1080/14656566.2018.1551362 (2019).

2 Raskin, P. Sodium-glucose cotransporter inhibition: therapeutic potential for the treatment of type 2 diabetes mellitus. *Diabetes Metab Res Rev* **29**, 347-356, doi:10.1002/dmrr.2403 (2013).

3 Zinman, B. *et al.* Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. *N Engl J Med*, doi:10.1056/NEJMoa1504720 (2015).

4 Wanner, C. *et al.* Empagliflozin and Progression of Kidney Disease in Type 2 Diabetes. *N Engl J Med* **375**, 323-334, doi:10.1056/NEJMoa1515920 (2016).

5 Neal, B. *et al.* Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. *N Engl J Med* **377**, 644-657, doi:10.1056/NEJMoa1611925 (2017).

6 Wiviott, S. D. *et al.* Dapagliflozin and Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* **380**, 347-357, doi:10.1056/NEJMoa1812389 (2019).

7 Zelniker, T. A. *et al.* SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet* **393**, 31-39, doi:10.1016/S0140-6736(18)32590-X (2019).

8 Perkovic, V. *et al.* Canagliflozin and Renal Outcomes in Type 2 Diabetes and

Nephropathy. *N Engl J Med* **380**, 2295-2306, doi:10.1056/NEJMoa1811744 (2019).

9 Zhang, G., Darshi, M. & Sharma, K. The Warburg Effect in Diabetic Kidney Disease. *Semin Nephrol* **38**, 111-120, doi:10.1016/j.semnephrol.2018.01.002 (2018).

10 Liberti, M. V. & Locasale, J. W. The Warburg Effect: How Does it Benefit Cancer Cells? *Trends Biochem Sci* **41**, 211-218, doi:10.1016/j.tibs.2015.12.001 (2016).

11 Yin, F. & Cadenas, E. Mitochondria: the cellular hub of the dynamic coordinated network. *Antioxid Redox Signal* **22**, 961-964, doi:10.1089/ars.2015.6313 (2015).

12 Kim, H. S. *et al.* SIRT3 is a mitochondria-localized tumor suppressor required for maintenance of mitochondrial integrity and metabolism during stress. *Cancer Cell* **17**, 41-52, doi:10.1016/j.ccr.2009.11.023 (2010).

13 Bindu, S. *et al.* SIRT3 blocks myofibroblast differentiation and pulmonary fibrosis by preventing mitochondrial DNA damage. *Am J Physiol Lung Cell Mol Physiol* **312**, L68-L78, doi:10.1152/ajplung.00188.2016 (2017).

14 Chen, T. *et al.* Activation of SIRT3 by resveratrol ameliorates cardiac fibrosis and improves cardiac function via the TGF-beta/Smad3 pathway. *Am J Physiol Heart Circ Physiol* **308**, H424-434, doi:10.1152/ajpheart.00454.2014 (2015).

15 He, P. *et al.* SIRT3 prevents angiotensin II-induced renal tubular epithelial-mesenchymal transition by ameliorating oxidative stress and mitochondrial dysfunction. *Mol Cell Endocrinol* **460**, 1-13, doi:10.1016/j.mce.2017.04.027 (2018).

16 Kim, D. *et al.* Absence of Sirt3 aggravates cisplatin nephrotoxicity via enhanced renal tubular apoptosis and inflammation. *Mol Med Rep* **18**, 3665-3672, doi:10.3892/mmr.2018.9350 (2018).

17 Koyama, T. *et al.* SIRT3 attenuates palmitate-induced ROS production and inflammation in proximal tubular cells. *Free radical biology & medicine* **51**, 1258-1267, doi:10.1016/j.freeradbiomed.2011.05.028 (2011).

18 Tran, M. T. *et al.* PGC1alpha drives NAD biosynthesis linking oxidative metabolism to renal protection. *Nature* **531**, 528-532, doi:10.1038/nature17184 (2016).

19 Yadav, H. *et al.* Protection from obesity and diabetes by blockade of TGF-beta/Smad3 signaling. *Cell Metab* **14**, 67-79, doi:10.1016/j.cmet.2011.04.013 (2011).

20 Kong, X. *et al.* Sirtuin 3, a new target of PGC-1alpha, plays an important role in the suppression of ROS and mitochondrial biogenesis. *PLoS One* **5**, e11707, doi:10.1371/journal.pone.0011707 (2010).

21 Zhang, X. *et al.* PGC-1alpha/ERRalpha-Sirt3 Pathway Regulates DAergic Neuronal Death by Directly Deacetylating SOD2 and ATP Synthase beta. *Antioxid Redox Signal* **24**, 312-328, doi:10.1089/ars.2015.6403 (2016).

22 Vallon, V. Glucose transporters in the kidney in health and disease. *Pflugers Arch*,

doi:10.1007/s00424-020-02361-w (2020).

23 DeNicola, G. M. & Cantley, L. C. Cancer's Fuel Choice: New Flavors for a Picky Eater. *Mol Cell* **60**, 514-523, doi:10.1016/j.molcel.2015.10.018 (2015).

24 Sukumar, M., Roychoudhuri, R. & Restifo, N. P. Nutrient Competition: A New Axis of Tumor Immunosuppression. *Cell* **162**, 1206-1208, doi:10.1016/j.cell.2015.08.064 (2015).

25 Jiang, Y. S., Jiang, T., Huang, B., Chen, P. S. & Ouyang, J. Epithelial-mesenchymal transition of renal tubules: divergent processes of repairing in acute or chronic injury? *Med Hypotheses* **81**, 73-75, doi:10.1016/j.mehy.2013.03.020 (2013).

26 Kalluri, R. & Weinberg, R. A. The basics of epithelial-mesenchymal transition. *The Journal of clinical investigation* **119**, 1420-1428, doi:10.1172/JCI39104 (2009).

27 Blantz, R. C. Phenotypic characteristics of diabetic kidney involvement. *Kidney Int* **86**, 7-9, doi:10.1038/ki.2013.552 (2014).

28 Lan, R. *et al.* Mitochondrial Pathology and Glycolytic Shift during Proximal Tubule Atrophy after Ischemic AKI. *J Am Soc Nephrol*, doi:10.1681/ASN.2015020177 (2016).

29 Eklund, T., Wahlberg, J., Ungerstedt, U. & Hillered, L. Interstitial lactate, inosine and hypoxanthine in rat kidney during normothermic ischaemia and recirculation. *Acta Physiol Scand* **143**, 279-286, doi:10.1111/j.1748-1716.1991.tb09233.x (1991).

30 Fukuhara, Y. *et al.* Changes in activities and mRNA levels of glycolytic enzymes of ischemia-reperfused rat kidney. *Contrib Nephrol* **95**, 222-228, doi:10.1159/000420663 (1991).

31 Kang, H. M. *et al.* Defective fatty acid oxidation in renal tubular epithelial cells has a key role in kidney fibrosis development. *Nat Med* **21**, 37-46, doi:10.1038/nm.3762 (2015).

32 Ash, S. R. & Cuppage, F. E. Shift toward anaerobic glycolysis in the regenerating rat kidney. *Am J Pathol* **60**, 385-402 (1970).

33 Jensen, T. M. *et al.* Methylglyoxal is associated with changes in kidney function among individuals with screen-detected Type 2 diabetes mellitus. *Diabet Med* **33**, 1625-1631, doi:10.1111/dme.13201 (2016).

34 Fattah, H., Layton, A. & Vallon, V. How Do Kidneys Adapt to a Deficit or Loss in Nephron Number? *Physiology (Bethesda)* **34**, 189-197, doi:10.1152/physiol.00052.2018 (2019).

35 Asmar, A. *et al.* Extracellular Fluid Volume Expansion Uncovers a Natriuretic Action of GLP-1: A Functional GLP-1-Renal Axis in Man. *J Clin Endocrinol Metab* **104**, 2509-2519, doi:10.1210/jc.2019-00004 (2019).

36 Bhargava, P. & Schnellmann, R. G. Mitochondrial energetics in the kidney. *Nat Rev Nephrol* **13**, 629-646, doi:10.1038/nrneph.2017.107 (2017).

37 Srivastava, S. P. *et al.* SIRT3 deficiency leads to induction of abnormal glycolysis in diabetic kidney with fibrosis. *Cell Death Dis* **9**, 997, doi:10.1038/s41419-018-1057-0

(2018).

38 Hamabe, A. *et al.* Role of pyruvate kinase M2 in transcriptional regulation leading to epithelial-mesenchymal transition. *Proc Natl Acad Sci U S A* **111**, 15526-15531, doi:10.1073/pnas.1407717111 (2014).

39 Koya, D. & King, G. L. Protein kinase C activation and the development of diabetic complications. *Diabetes* **47**, 859-866, doi:10.2337/diabetes.47.6.859 (1998).

40 Sugimoto, H., Grahovac, G., Zeisberg, M. & Kalluri, R. Renal fibrosis and glomerulosclerosis in a new mouse model of diabetic nephropathy and its regression by bone morphogenic protein-7 and advanced glycation end product inhibitors. *Diabetes* **56**, 1825-1833, doi:10.2337/db06-1226 (2007).

41 Mizunuma, Y., Kanasaki, K. & etc. CD-1db/db mice: a novel type 2 diabetic mouse model with progressive kidney fibrosis. *J Diabetes Investig* **In Press** (2020).

42 Kanasaki, K. *et al.* Linagliptin-mediated DPP-4 inhibition ameliorates kidney fibrosis in streptozotocin-induced diabetic mice by inhibiting endothelial-to-mesenchymal transition in a therapeutic regimen. *Diabetes* **63**, 2120-2131, doi:10.2337/db13-1029 (2014).

43 Shi, S. *et al.* Interactions of DPP-4 and integrin beta1 influences endothelial-to-mesenchymal transition. *Kidney Int* **88**, 479-489, doi:10.1038/ki.2015.103 (2015).

44 Li, J. *et al.* FGFR1 is critical for the anti-endothelial mesenchymal transition effect of N-acetyl-seryl-aspartyl-lysyl-proline via induction of the MAP4K4 pathway. *Cell Death & Disease* **in Press** (2017).

45 Takagaki, Y. *et al.* Dipeptidyl peptidase-4 plays a pathogenic role in BSA-induced kidney injury in diabetic mice. *Sci Rep* **9**, 7519, doi:10.1038/s41598-019-43730-5 (2019).

46 Li, J. *et al.* Renal protective effects of empagliflozin via inhibition of EMT and aberrant glycolysis in proximal tubules. *JCI Insight* **5**, doi:10.1172/jci.insight.129034 (2020).

47 Wong, N., Ojo, D., Yan, J. & Tang, D. PKM2 contributes to cancer metabolism. *Cancer Lett* **356**, 184-191, doi:10.1016/j.canlet.2014.01.031 (2015).

48 Qi, W. *et al.* Pyruvate kinase M2 activation may protect against the progression of diabetic glomerular pathology and mitochondrial dysfunction. *Nat Med* **23**, 753-762, doi:10.1038/nm.4328 (2017).

49 Mandel, L. J. Metabolic substrates, cellular energy production, and the regulation of proximal tubular transport. *Annu Rev Physiol* **47**, 85-101, doi:10.1146/annurev.ph.47.030185.000505 (1985).

50 Wirthensohn, G. & Guder, W. G. Renal substrate metabolism. *Physiol Rev* **66**, 469-497, doi:10.1152/physrev.1986.66.2.469 (1986).

51 Hato, T. *et al.* Novel application of complimentary imaging techniques to examine in vivo glucose metabolism in the kidney. *Am J Physiol Renal Physiol*, ajprenal 00535 02015,

doi:10.1152/ajprenal.00535.2015 (2016).

52 Kim, J., Devalaraja-Narashimha, K. & Padanilam, B. J. TIGAR regulates glycolysis in ischemic kidney proximal tubules. *Am J Physiol Renal Physiol* **308**, F298-308, doi:10.1152/ajprenal.00459.2014 (2015).

53 Lee, H. *et al.* Increased mitochondrial activity in renal proximal tubule cells from young spontaneously hypertensive rats. *Kidney Int* **85**, 561-569, doi:10.1038/ki.2013.397 (2014).

54 Ding, H. *et al.* Inhibiting aerobic glycolysis suppresses renal interstitial fibroblast activation and renal fibrosis. *Am J Physiol Renal Physiol* **313**, F561-F575, doi:10.1152/ajprenal.00036.2017 (2017).

55 Salmond, R. J. mTOR Regulation of Glycolytic Metabolism in T Cells. *Front Cell Dev Biol* **6**, 122, doi:10.3389/fcell.2018.00122 (2018).

56 Mostov, K. E. mTOR is out of control in polycystic kidney disease. *Proc Natl Acad Sci U S A* **103**, 5247-5248, doi:10.1073/pnas.0601352103 (2006).

57 Ibraghimov-Beskrovnaya, O. & Natoli, T. A. mTOR signaling in polycystic kidney disease. *Trends Mol Med* **17**, 625-633, doi:10.1016/j.molmed.2011.06.003 (2011).

58 Torres, J. A. *et al.* Ketosis Ameliorates Renal Cyst Growth in Polycystic Kidney Disease. *Cell Metab* **30**, 1007-1023 e1005, doi:10.1016/j.cmet.2019.09.012 (2019).

59 Rowe, I. *et al.* Defective glucose metabolism in polycystic kidney disease identifies a new therapeutic strategy. *Nat Med* **19**, 488-493, doi:10.1038/nm.3092 (2013).

60 Riwanto, M. *et al.* Inhibition of Aerobic Glycolysis Attenuates Disease Progression in Polycystic Kidney Disease. *PLoS One* **11**, e0146654, doi:10.1371/journal.pone.0146654 (2016).

61 Chiaravalli, M. *et al.* 2-Deoxy-d-Glucose Ameliorates PKD Progression. *J Am Soc Nephrol* **27**, 1958-1969, doi:10.1681/ASN.2015030231 (2016).

62 Li, A. *et al.* Rapamycin treatment dose-dependently improves the cystic kidney in a new ADPKD mouse model via the mTORC1 and cell-cycle-associated CDK1/cyclin axis. *J Cell Mol Med* **21**, 1619-1635, doi:10.1111/jcmm.13091 (2017).

63 Lund, T. M., Ploug, K. B., Iversen, A., Jensen, A. A. & Jansen-Olesen, I. The metabolic impact of beta-hydroxybutyrate on neurotransmission: Reduced glycolysis mediates changes in calcium responses and KATP channel receptor sensitivity. *J Neurochem* **132**, 520-531, doi:10.1111/jnc.12975 (2015).

64 Shen, Y. *et al.* Tubule-derived lactate is required for fibroblast activation in acute kidney injury. *Am J Physiol Renal Physiol* **318**, F689-F701, doi:10.1152/ajprenal.00229.2019 (2020).

65 Gerich, J. E. Role of the kidney in normal glucose homeostasis and in the hyperglycaemia of diabetes mellitus: therapeutic implications. *Diabet Med* **27**, 136-142,

doi:10.1111/j.1464-5491.2009.02894.x (2010).

66 Ferrannini, E., Veltkamp, S. A., Smulders, R. A. & Kadokura, T. Renal glucose handling: impact of chronic kidney disease and sodium-glucose cotransporter 2 inhibition in patients with type 2 diabetes. *Diabetes Care* **36**, 1260-1265, doi:10.2337/dc12-1503 (2013).

67 Bendon, R. W. & Hug, G. Glycogen accumulation in the pars recta of the proximal tubule in Fanconi syndrome. *Pediatr Pathol* **6**, 411-429, doi:10.3109/15513818609041556 (1986).

68 Chen, Y. T., Coleman, R. A., Scheinman, J. I., Kolbeck, P. C. & Sidbury, J. B. Renal disease in type I glycogen storage disease. *N Engl J Med* **318**, 7-11, doi:10.1056/NEJM198801073180102 (1988).

69 Reitsma-Bierens, W. C. Renal complications in glycogen storage disease type I. *Eur J Pediatr* **152 Suppl 1**, S60-62, doi:10.1007/bf02072091 (1993).

70 Mihout, F. *et al.* Acute metabolic acidosis in a GLUT2-deficient patient with Fanconi-Bickel syndrome: new pathophysiology insights. *Nephrol Dial Transplant* **29 Suppl 4**, iv113-116, doi:10.1093/ndt/gfu018 (2014).

71 Imai, S. I. & Guarente, L. It takes two to tango: NAD(+) and sirtuins in aging/longevity control. *NPJ Aging Mech Dis* **2**, 16017, doi:10.1038/npjamd.2016.17 (2016).

72 Kincaid, B. & Bossy-Wetzel, E. Forever young: SIRT3 a shield against mitochondrial meltdown, aging, and neurodegeneration. *Front Aging Neurosci* **5**, 48, doi:10.3389/fnagi.2013.00048 (2013).

73 Jia, Y. *et al.* Dapagliflozin Aggravates Renal Injury via Promoting Gluconeogenesis in db/db Mice. *Cell Physiol Biochem* **45**, 1747-1758, doi:10.1159/000487783 (2018).

74 Finley, L. W. *et al.* SIRT3 opposes reprogramming of cancer cell metabolism through HIF1alpha destabilization. *Cancer Cell* **19**, 416-428, doi:10.1016/j.ccr.2011.02.014 (2011).

75 Palmirotta, R. *et al.* Sirtuins and Cancer: Role in the Epithelial-Mesenchymal Transition. *Oxid Med Cell Longev* **2016**, 3031459, doi:10.1155/2016/3031459 (2016).

76 Higgins, D. F. *et al.* Hypoxia promotes fibrogenesis in vivo via HIF-1 stimulation of epithelial-to-mesenchymal transition. *The Journal of clinical investigation* **117**, 3810-3820, doi:10.1172/JCI30487 (2007).

77 Sun, S. *et al.* Hypoxia-inducible factor-1alpha induces Twist expression in tubular epithelial cells subjected to hypoxia, leading to epithelial-to-mesenchymal transition. *Kidney Int* **75**, 1278-1287, doi:10.1038/ki.2009.62 (2009).

78 Luo, W. *et al.* Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. *Cell* **145**, 732-744, doi:10.1016/j.cell.2011.03.054 (2011).

79 Wang, H. J. *et al.* JMJD5 regulates PKM2 nuclear translocation and reprograms HIF-

1alpha-mediated glucose metabolism. *Proc Natl Acad Sci U S A* **111**, 279-284, doi:10.1073/pnas.1311249111 (2014).

80 Gao, X., Wang, H., Yang, J. J., Liu, X. & Liu, Z. R. Pyruvate kinase M2 regulates gene transcription by acting as a protein kinase. *Mol Cell* **45**, 598-609, doi:10.1016/j.molcel.2012.01.001 (2012).

81 Zhang, Z. *et al.* PKM2, function and expression and regulation. *Cell Biosci* **9**, 52, doi:10.1186/s13578-019-0317-8 (2019).

82 Han, J. *et al.* Interleukin-6 stimulates aerobic glycolysis by regulating PFKFB3 at early stage of colorectal cancer. *Int J Oncol* **48**, 215-224, doi:10.3892/ijo.2015.3225 (2016).

83 Yin, X. *et al.* Hexokinase 2 couples glycolysis with the profibrotic actions of TGF-beta. *Sci Signal* **12**, doi:10.1126/scisignal.aax4067 (2019).

84 Wang, S. W. & Sun, Y. M. The IL-6/JAK/STAT3 pathway: potential therapeutic strategies in treating colorectal cancer (Review). *Int J Oncol* **44**, 1032-1040, doi:10.3892/ijo.2014.2259 (2014).

85 Li, B. & Huang, C. Regulation of EMT by STAT3 in gastrointestinal cancer (Review). *Int J Oncol* **50**, 753-767, doi:10.3892/ijo.2017.3846 (2017).

86 Li, M. *et al.* STAT3 regulates glycolysis via targeting hexokinase 2 in hepatocellular carcinoma cells. *Oncotarget* **8**, 24777-24784, doi:10.18632/oncotarget.15801 (2017).

87 Zhang, C. *et al.* STAT3 Activation-Induced Fatty Acid Oxidation in CD8(+) T Effector Cells Is Critical for Obesity-Promoted Breast Tumor Growth. *Cell Metab* **31**, 148-161 e145, doi:10.1016/j.cmet.2019.10.013 (2020).

88 Demaria, M. *et al.* A STAT3-mediated metabolic switch is involved in tumour transformation and STAT3 addiction. *Aging (Albany NY)* **2**, 823-842, doi:10.18632/aging.100232 (2010).

89 Pawlus, M. R., Wang, L., Murakami, A., Dai, G. & Hu, C. J. STAT3 or USF2 contributes to HIF target gene specificity. *PLoS One* **8**, e72358, doi:10.1371/journal.pone.0072358 (2013).

90 Demaria, M. & Poli, V. PKM2, STAT3 and HIF-1alpha: The Warburg's vicious circle. *JAKSTAT* **1**, 194-196, doi:10.4161/jkst.20662 (2012).

91 Ferrannini, E. *et al.* Shift to Fatty Substrate Utilization in Response to Sodium-Glucose Cotransporter 2 Inhibition in Subjects Without Diabetes and Patients With Type 2 Diabetes. *Diabetes* **65**, 1190-1195, doi:10.2337/db15-1356 (2016).

92 Kanasaki, K., Taduri, G. & Koya, D. Diabetic nephropathy: the role of inflammation in fibroblast activation and kidney fibrosis. *Front Endocrinol (Lausanne)* **4**, 7, doi:10.3389/fendo.2013.00007 (2013).

93 Loeffler, I. & Wolf, G. Epithelial-to-Mesenchymal Transition in Diabetic Nephropathy: Fact

or Fiction? *Cells* **4**, 631-652, doi:10.3390/cells4040631 (2015).

94 Fragiadaki, M. & Mason, R. M. Epithelial-mesenchymal transition in renal fibrosis - evidence for and against. *Int J Exp Pathol* **92**, 143-150, doi:10.1111/j.1365-2613.2011.00775.x (2011).

95 Kramann, R. & Humphreys, B. D. Kidney pericytes: roles in regeneration and fibrosis. *Semin Nephrol* **34**, 374-383, doi:10.1016/j.semnephrol.2014.06.004 (2014).

96 Sano, M. A new class of drugs for heart failure: SGLT2 inhibitors reduce sympathetic overactivity. *J Cardiol* **71**, 471-476, doi:10.1016/j.jjcc.2017.12.004 (2018).

97 Scheen, A. J. Effects of glucose-lowering agents on surrogate endpoints and hard clinical renal outcomes in patients with type 2 diabetes. *Diabetes Metab* **45**, 110-121, doi:10.1016/j.diabet.2018.10.003 (2019).

98 Cherney, D. Z. *et al.* Renal hemodynamic effect of sodium-glucose cotransporter 2 inhibition in patients with type 1 diabetes mellitus. *Circulation* **129**, 587-597, doi:10.1161/CIRCULATIONAHA.113.005081 (2014).

99 Hasegawa, K. *et al.* Renal tubular Sirt1 attenuates diabetic albuminuria by epigenetically suppressing Claudin-1 overexpression in podocytes. *Nat Med* **19**, 1496-1504, doi:10.1038/nm.3363 (2013).

100 Nangaku, M. Chronic hypoxia and tubulointerstitial injury: a final common pathway to end-stage renal failure. *Journal of the American Society of Nephrology : JASN* **17**, 17-25, doi:10.1681/ASN.2005070757 (2006).

101 Maki, T. *et al.* Amelioration of diabetic nephropathy by SGLT2 inhibitors independent of its glucose-lowering effect: A possible role of SGLT2 in mesangial cells. *Sci Rep* **9**, 4703, doi:10.1038/s41598-019-41253-7 (2019).

102 Cassis, P. *et al.* SGLT2 inhibitor dapagliflozin limits podocyte damage in proteinuric nondiabetic nephropathy. *JCI Insight* **3**, doi:10.1172/jci.insight.98720 (2018).

103 Scafoglio, C. R. *et al.* Sodium-glucose transporter 2 is a diagnostic and therapeutic target for early-stage lung adenocarcinoma. *Sci Transl Med* **10**, doi:10.1126/scitranslmed.aat5933 (2018).

104 Cherney, D. Z. I. *et al.* Effects of empagliflozin on the urinary albumin-to-creatinine ratio in patients with type 2 diabetes and established cardiovascular disease: an exploratory analysis from the EMPA-REG OUTCOME randomised, placebo-controlled trial. *Lancet Diabetes Endocrinol* **5**, 610-621, doi:10.1016/S2213-8587(17)30182-1 (2017).

Cardiovascular and renal outcomes in clinical trials with SGLT2 inhibitors

	EMPA-REG OUTCOME[3,4]	CANVAS Program[5]	DECLARE-TIMI58[6]	CREDENCE[8]
SGLT2 inhibitor	Empagliflozin, 10 or 25 mg	Canagliflozin, 100-300 mg	Dapagliflozin, 10 mg	Canagliflozin, 100 mg
Follow up (years)	3.1	3.6	4.2	2.62
Patients (n), (age)	7,020 (63.2)	10,142 (63.3)	17,160 (63.9)	4,410 (63.0)
% patients with ASCVD	>99	65.6	40.6	50.4 (all cardiovascular disease)
Inclusion criteria	All patients with established cardiovascular disease. eGFR >30	HbA1c ≥7.0 and ≤10.5 ≥30 years old with a history of symptomatic ASCVD or, ≥50 years old with two or more of the risk factors for cardiovascular disease. eGFR >30	Either established ASCVD or multiple risk factors for ASCVD. eGFR >60	Albuminuria >300 to 500 mg/gCr eGFR 30-90
Primary outcome	Composite of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke	Composite of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke	MACE* composite of cardiovascular death, hospitalization for heart failure**	Composite of end-stage kidney disease, a doubling of the serum creatinine level, or death from renal or cardiovascular causes
Primary endpoint	0.86 (0.74-0.99) p = 0.04	0.86 (0.75-0.97) p = 0.02	* 0.93 (0.84-1.03) p = 0.17 ** 0.83 (0.73-0.95) p = 0.005	0.70 (0.59-0.82) p = 0.00001
Renal outcome	Incident or worsening nephropathy*	Progression of albuminuria# Regression of albuminuria## Composites of 40% decrease (eGFR), renal death, or requirement for renal replacement therapy###	Composite of a sustained decrease of 40% or more in eGFR to < 60, new end-stage renal disease, or death from renal or cardiovascular causes	Composite of end-stage kidney disease, a doubling of the creatinine level, or ranal death
Renal endpoint	0.61 (0.53-0.70) p < 0.001	# 0.73 (0.67-0.79 ## 1.70 (1.51-1.91) ### 0.60 (0.47-0.77)	0.76 (0.64-0.87	0.66 (0.53-0.81) p < 0.001

Table1

*defined as progression to macroalbuminuria; a doubling of the serum creatinine level, accompanied by an eGFR of ≤ 45 ml per minute per 1.73 m^2 ,as calculated by the MDRD formula; the initiation of renal-replacement therapy; or death from renal disease
ASCVD: atherosclerotic cardiovascular disease

SGLT2 actions

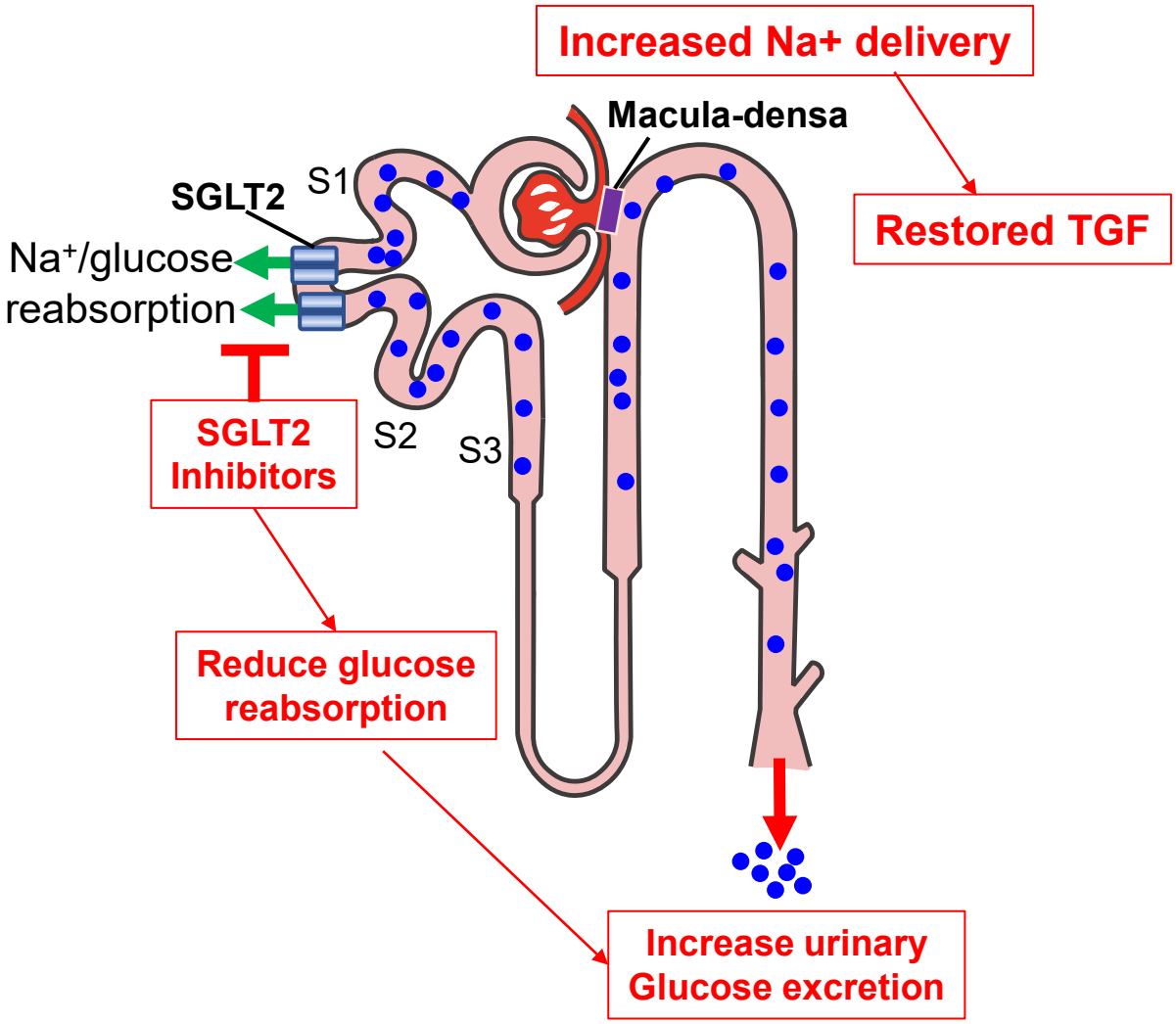

FIG 1

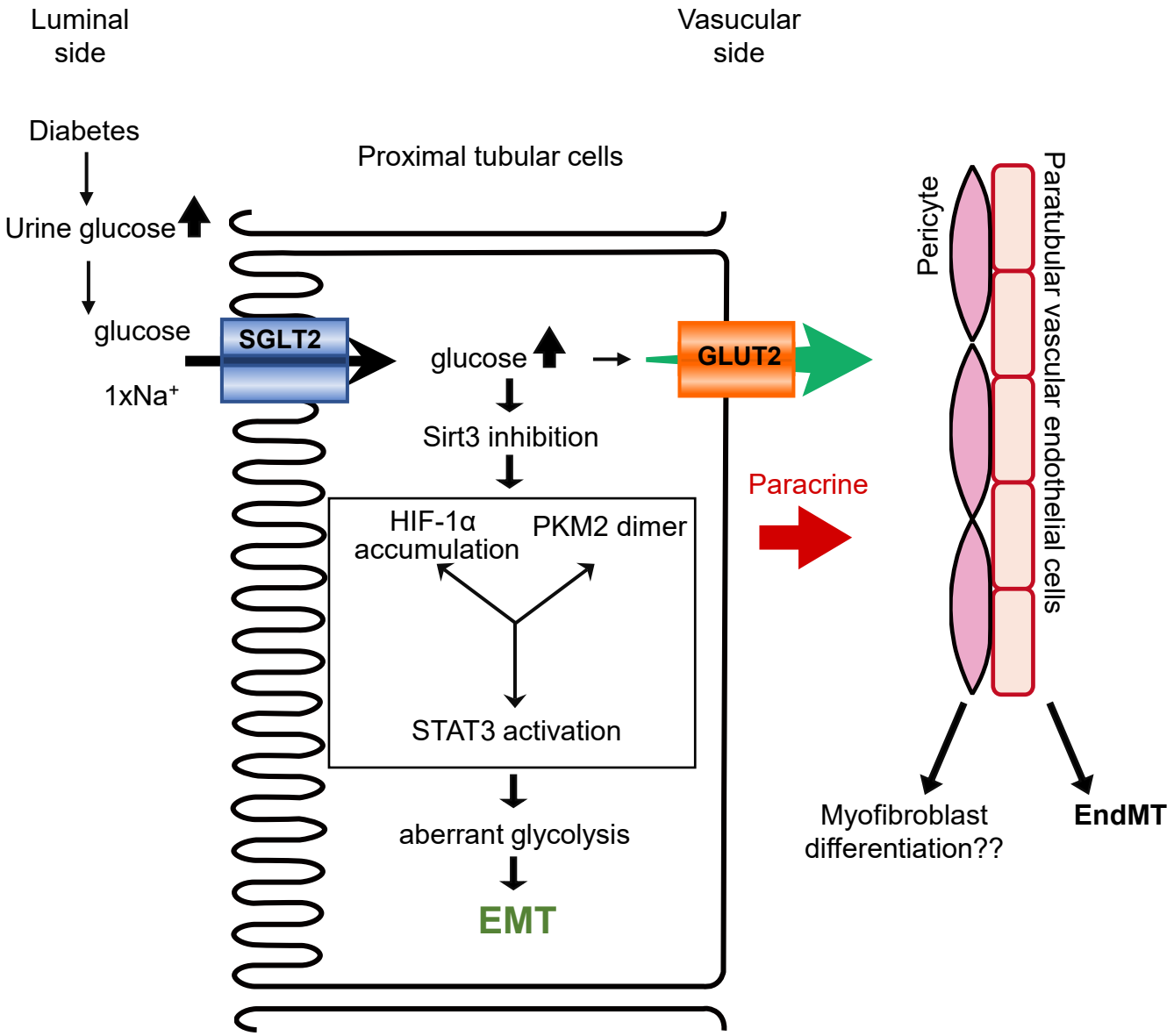
Luminal side
Vasucular side
Diabetes
Urine glucose
glucose
1xNa+
SGLT2
Proximal tubular cells
glucose
GLUT2
Sirt3 inhibition
HIF-1α accumulation
PKM2 dimer
STAT3 activation
aberrant glycolysis
EMT
Paracrine
Pericyte
Paratubular vascular endothelial cells
Myofibroblast differentiation??
EndMT

FIG 2

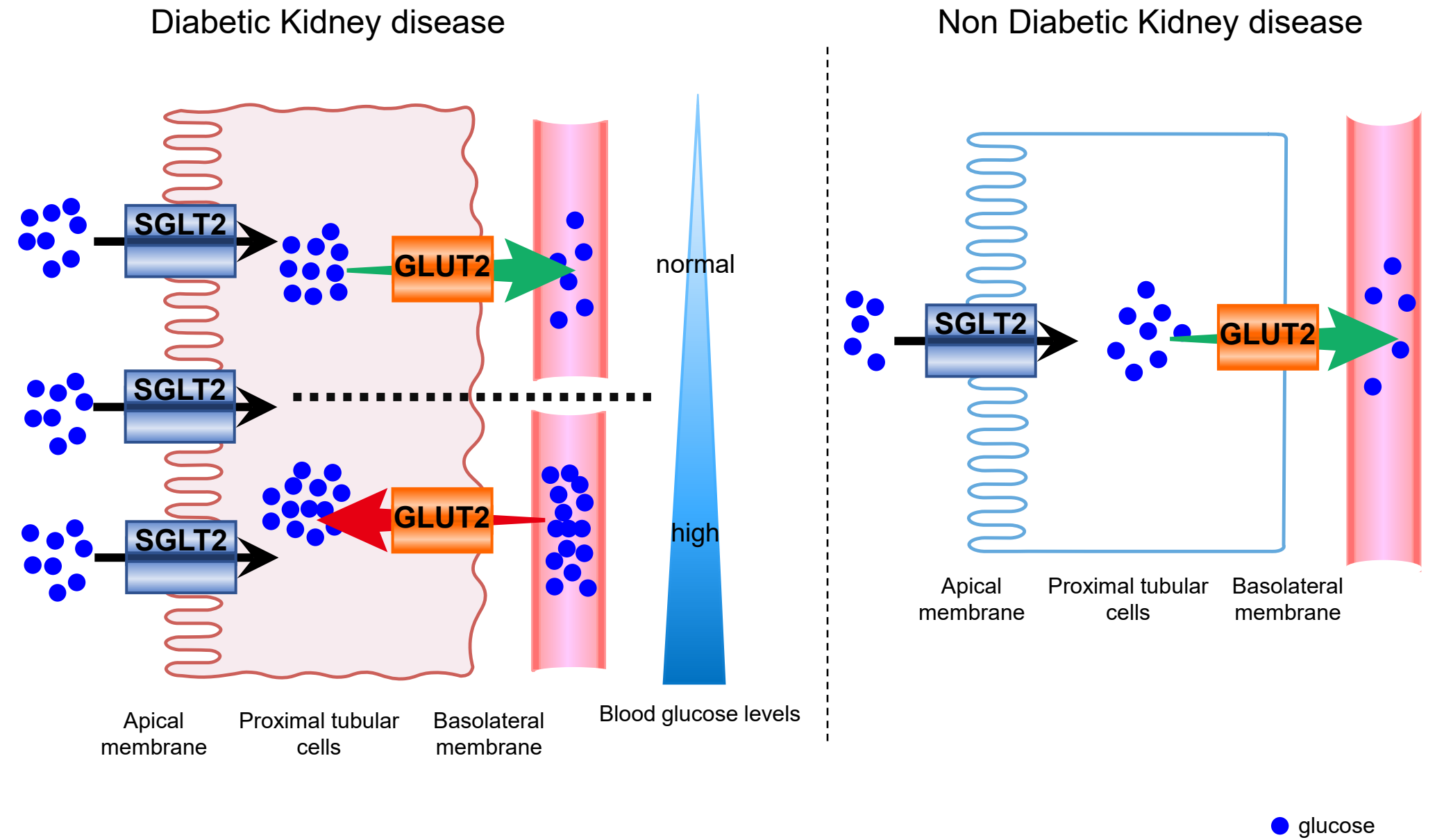
Diabetic Kidney disease
Non Diabetic Kidney disease
SGLT2
SGLT2
SGLT2
GLUT2
GLUT2
normal
high
Apical membrane
Proximal tubular cells
Basolateral membrane
Blood glucose levels
SGLT2
GLUT2
Apical membrane
Proximal tubular cells
Basolateral membrane
glucose

FIG 3

Potential Renoprotection

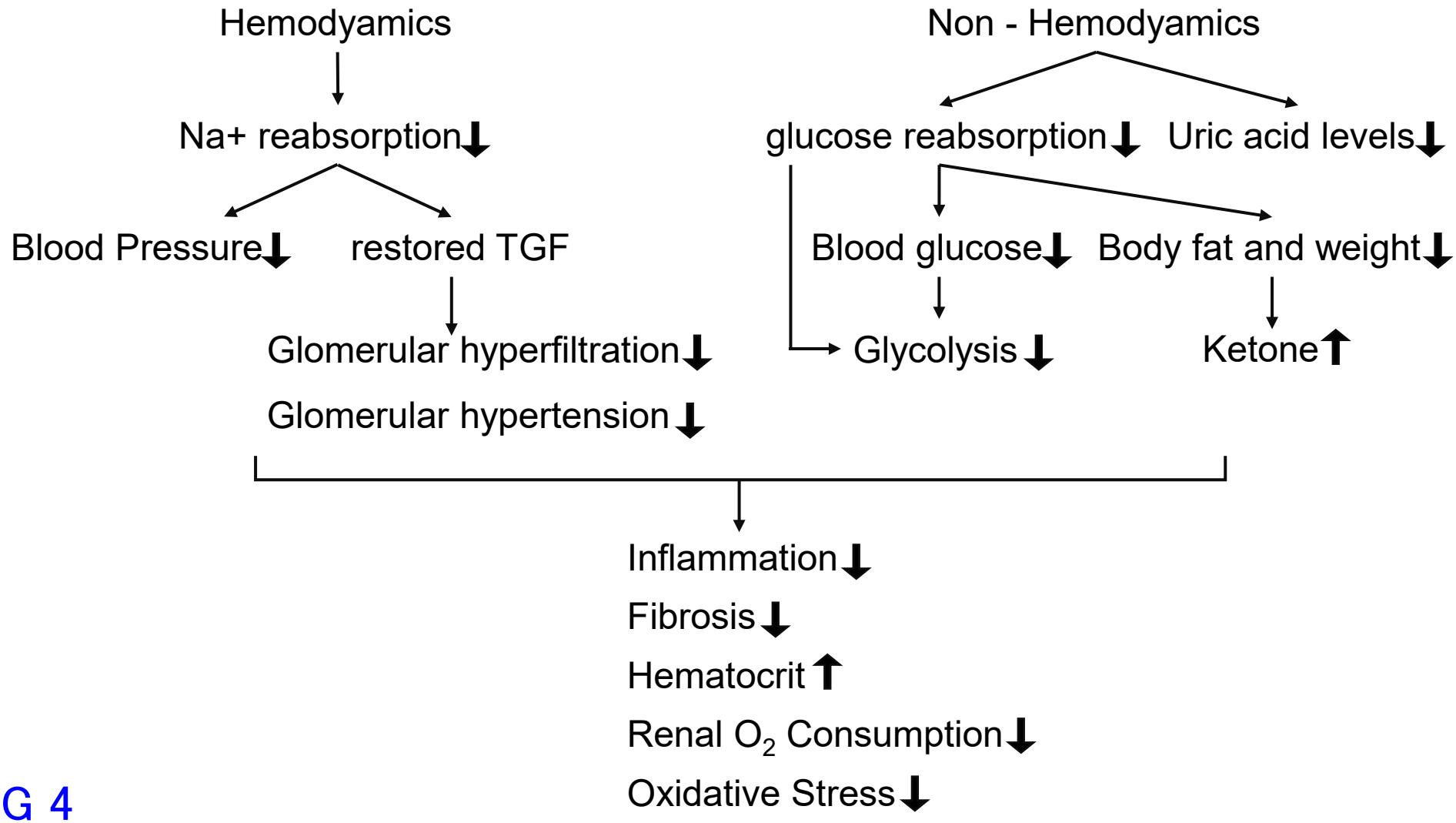

FIG 4