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New Tubulocentric Insights for Diabetic Nephropathy: From Pathophysiology to Treatment

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Abstract

The prevalence of diabetes is increasing worldwide, and one of the most important complications, diabetic nephropathy, constitutes a significant global health care and socioeconomic burden. Glomerular dysfunction is a major factor in the development and progression of diabetic nephropathy. However, emerging evidence suggests that tubular damage also plays an important role in the pathogenesis of diabetic nephropathy. This tubulocentric view shifts the focus markedly from glomeruli to proximal tubules, which might have an important role as a trigger or a driver in the early development and progression of diabetic nephropathy. Accordingly, numerous studies have focused on several different tubular damage markers that are clinically indicated as potential biomarkers for the early detection of diabetic nephropathy. Furthermore, these findings are relevant for identifying therapeutics for diabetic nephropathy that target the proximal tubules. This review outlines new tubulocentric insights into diabetic nephropathy, from pathophysiological mechanisms to diagnostic and therapeutic approaches.

Keywords: biomarkers, diabetic nephropathy, glomerulus, proximal tubules, SGLT2 inhibitors

1. Introduction

Diabetes is often accompanied by chronic kidney disease (CKD) and accounts for more than half of the cause of end-stage renal disease (ESRD) and dialysis [1]. With the increasing prevalence of diabetes and high morbidity and mortality, its complications such as diabetic nephropathy impose great burden on individuals with diabetes and their society as well [2]. It is unclear whether the glomerulus or tubules are more important in the development and

progression of diabetic nephropathy. The phenomenon of glomerulosclerosis led to an interest in the glomerulus as the primary site of injury in diabetic nephropathy. Indeed, changes in glomerular structure, such as glomerular basement membrane thickening, mesangial expansion, and nodular/global glomerulosclerosis, are key findings for the diagnosis of diabetic nephropathy and other forms of glomerulonephropathy [3]. Although changes in the glomerulus in diabetic neuropathy undoubtedly occur, there is growing evidence to suggest a prominent role for the proximal tubules as triggers or drivers of diabetic nephropathy. Indeed, this evidence provides a new perspective on the natural course and pathophysiology of diabetic nephropathy. These novel insights also provide new opportunities for diagnostic and therapeutic progress through targeting the proximal tubules in diabetic nephropathy. This review provides an outline of diabetic nephropathy, from the underlying pathophysiological mechanism to diagnostic and therapeutic approaches, based on a tubulocentric perspective.

2. Natural course of diabetic nephropathy: old and new

It is very important to understand the natural course of disease to ensure that technical advances are fully exploited. However, the natural course of diabetic nephropathy is complex and depends on several factors, such as the clinical treatments used, and the race, type of diabetes, and comorbidities of the patient. Therefore, it is difficult to treat and prevent diabetic nephropathy. Current treatments include renin-angiotensin system (RAS) blockade, antihypertensives, glycemic control, and correction of dyslipidemia for the management of diabetic complications. Diabetes mellitus (DM) has a long history and was first described in 1552 BC; it has long been recognized as a socioeconomic burden [4]. Diabetes is regarded as a metabolic derangement and is closely related to renal dysfunction, as a microvascular complication [5]. Classically, diabetic nephropathy has a five-stage natural history: hyperfiltration, silent nephropathy, incipient nephropathy (microalbuminuric stage), overt nephropathy (macroalbuminuric stage), and ESRD [6, 7]. These five stages are almost exclusively applied to discussions of type 1 diabetes since the precise onset of disease in type 2 diabetes is not known. This natural course of diabetic nephropathy has served as a basis for clinical practice, and there are ongoing efforts to reduce albuminuria in patients with type 2 diabetes and renal dysfunction. Most adult cases of diabetes are type 2, and it is critical to delineate the progress of this disease. Type 2 diabetes differs from type 1 diabetes in several aspects. First, it is impossible to determine disease onset in type 2 diabetes. Second, in many cases, hypertension and albuminuria commonly accompany type 2 diabetes. Third, microalbuminuria has a lower predictive value for renal dysfunction because of the high mortality rate caused by cardiovascular disease [7, 8]. Recently, a new paradigm was suggested for exploring the natural course of diabetic nephropathy in the context of microalbuminuria and the nonclassical form of the disease.

2.1. Microalbuminuria: moderately increased albuminuria

Microalbuminuria is a robust indicator of the onset and progression of diabetic nephropathy, and it is assessed by reference to serum creatinine levels or the estimated glomerular filtration rate (eGFR). However, microalbuminuria has some major limitations as a predictor of renal dysfunction [9]. Albuminuria measurements can be imprecise and vary widely according to

the assay method used, the time of urine collection, and the presence of clinical conditions such as fever, urinary tract infection, and congestive heart failure, as well as by exercise status [10]. Microalbuminuria was originally considered a subcategory denoted by an albuminuria level of 30–299 mg/day in a 24-h urine sample or 30–299 mg/g creatinine in a spot urine sample. Recently, normoalbuminuria and microalbuminuria were replaced by "normal to mildly increased albuminuria" and "moderately increased albuminuria," respectively, because albuminuria is directly related to all-cause mortality, cardiovascular mortality, and renal dysfunction, even in patients with normoalbuminuria and microalbuminuria [11, 12] Furthermore, microalbuminuria shows dynamic characteristics (**Figure 1**), being a transient state that can progress to macroalbuminuria or regress to normoalbuminuria [13, 14]. A 7-year prospective study performed by the EURODIAB IDDM Complications Study Group in 352 type 1 diabetic patients showed that ~14% progressed to macroalbuminuria, 35% remained in a microalbuminuric state, and 51% regressed to normoalbuminuria [13]. The Joslin study reported that 58% of 386 type 1 diabetic patients with persistent microalbuminuria regressed to normoalbuminuria [14]. A better glycemic control contributes to the regression of microalbuminuria, and almost half of patients with microalbuminuria can regress to normoalbuminuria, as evidenced by the above two large studies. Although microalbuminuria is caused by glomerular injury, recent research has focused on the role of tubular dysfunction in albuminuria in type 1 diabetes. The Second Joslin Kidney Study reported that kidney injury molecule-1 (KIM-1) and N-acetyl-β-D-glucosaminidase (NAG) levels were important for predicting the regression of microalbuminuria [15]. This finding supports the theory that tubular injury plays a significant role in the progression of renal complications in type 1 diabetes. This pattern of microalbuminuria regression could also be applicable to type 2 diabetes, although no concrete evidence for this has been reported.
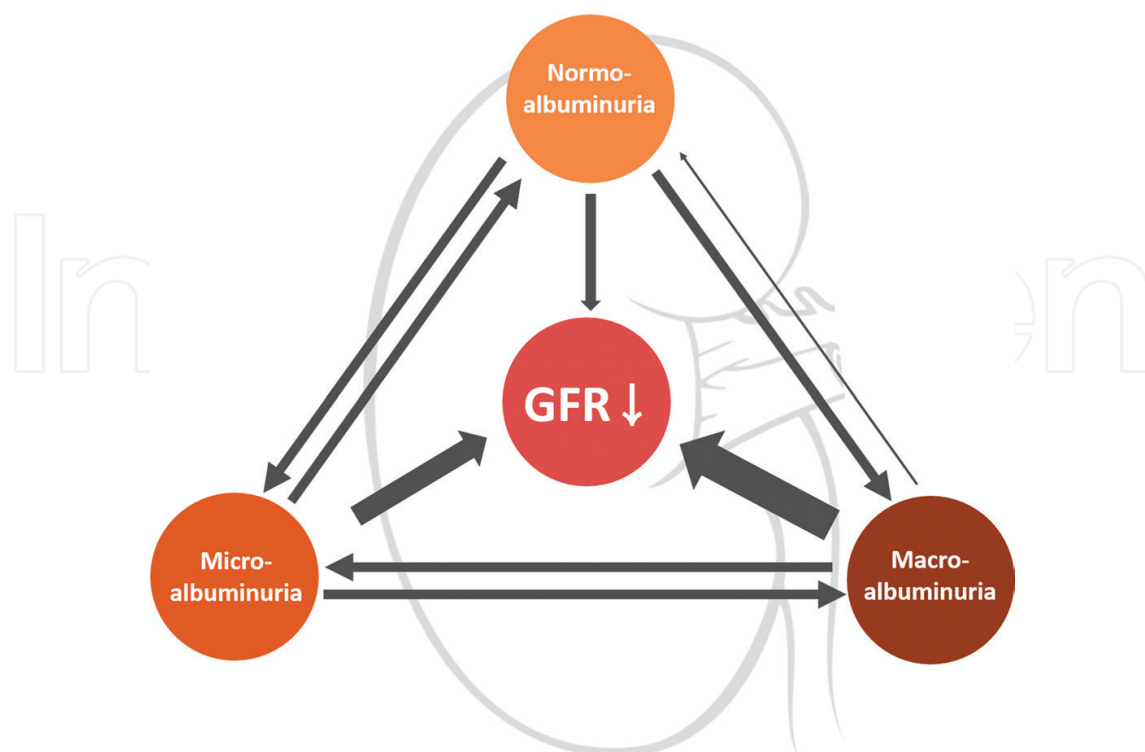

Figure 1. A triangle concept toward kidney function. eGFR, estimated glomerular filtration rate.

In 216 Japanese patients with type 2 diabetes and microalbuminuria, the regression rate to a normoalbuminuric state was ~50%, where regression was associated with a RAS-blocking agent, a better glycemic control, and a tight control of blood pressure [9]. However, none of 60 patients with type 2 microalbuminuric diabetic nephropathy regressed to normoalbuminuria in an African-American population [16]. This suggests that there are racial differences in changes in microalbuminuria status in type 2 diabetes, and further studies are needed to explore the role of genetic predisposition and race. A recent study suggested that macroalbuminuria and microalbuminuria can regress to microalbuminuria or normoalbuminuria, respectively [17]. In the FinnDiane study, 23.4% of 475 type 1 diabetic patients with macroalbuminuria regressed to a lower categorical albuminuric state and 2.5% regressed to normoalbuminuria, although the statistical power was low [17]. Such regression would improve the cardiovascular prognosis and all-cause mortality. Previous studies proposed that the regression of microalbuminuria contributes to a reduction in renal or cardiovascular risk in type 2 diabetic and hypertensive patients [18, 19]. These data suggest that it is necessary to treat diabetic patients with some degree of albuminuria to regress the albuminuria.

2.2. Nonalbumin proteinuria

Protein in urine comprises albumin (40%) and nonalbumin proteins (NAPs; 60%). A third of NAPs are low-molecular weight proteins (LMWP), such as light-chain immunoglobulins (20%), and two-thirds are Tamm-Horsfall mucoproteins produced by the distal tubules [20, 21]. The proportional contribution of albumin attributes to be largely more variable at lower levels of proteinuria, and NAPs are important when assessing proteinuria as a biomarker of renal tubular damage [22]. Nonalbumin proteinuria can be defined as an albumin excretion rate (AER) of < 30 mg/24 h with a protein excretion rate (PER) of >149 mg/24 h. Nonalbumin proteinuria can be quantified in random spot urine samples using the following formula: NAP-to-creatinine ratio (NAPCR) = protein creatinine ratio (PCR) − albumin creatinine ratio (ACR). Because albuminuria tests could miss up to 40% of females and 30.8% of males in the general population with gross proteinuria, NAP levels should be checked to accurately assess renal damage [21]. Our laboratory reported that urinary NAPCR had a significant association with the decline in eGFR of 237 type 2 diabetic patients with preserved kidney function and normoalbuminuria [23]. In addition, NAP was related to tubular biomarkers such as KIM-1, neutrophil gelatinase-associated lipocalin (NGAL), and liver-type fatty acid-binding protein (L-FABP) in early type 2 diabetic nephropathy patients with preserved kidney function (eGFR ≥60 mL/min/1.73 m^2) [24]. Moreover, NAPCR could serve a simpler and a more practical marker for assessing the progression of renal dysfunction compared with laboratory urinary biomarkers, such as KIM-1, NGAL, and L-FABP [25]. In the future, it will be necessary to study NAP in diabetic nephropathy to discover novel processes in and investigate the course of the disease.

2.3. Normoalbuminuric renal decline (NARD)

In general clinical practice, the eGFR should be calculated at least once a year to properly manage diabetic patients. Like albuminuria, eGFR has some major limitations for predicting renal dysfunction, because serum creatinine and cystatin C levels cannot be measured precisely and do

not reflect early changes in the kidney. However, currently, there are no available tools that are more powerful for assessing kidney function. Although the classic course of diabetic nephropathy involves sequential dysfunction of the kidney following albuminuria, recent epidemiologic data suggested the presence of normoalbuminuric diabetic nephropathy in some patients [26]. There is a close relationship between albuminuria and progressive dysfunction of the diabetic kidney. A recent Japanese study showed that a rapid decline in kidney function occurred in subjects with higher levels of ACR of ≥3000 mg/g creatinine in urine. In addition, the rate of annual decline in eGFR was doubled in macroalbuminuric versus normoalbuminuric diabetics for 9.2 years [11]. However, the focus should be on NARD with respect to early intervention strategies, because dipstick tests cannot reveal low levels of albuminuria or NAP. The UK Prospective Diabetes Study (UKPDS) reported that, among the patients who developed renal impairment during the study, 61% did not have albuminuria beforehand and 39% never developed albuminuria [27]. This suggests that distinct pathobiological mechanisms may underlie NARD and albuminuric renal decline. The prevalence of NARD was not low (20.5–63%) in several clinical trials performed in type 2 diabetic patients [26]. Interestingly, in another study, the prevalence of retinopathy was lower in the NARD group than in the albuminuric group, and patients with NARD had a shorter duration of diabetes [28]. This finding gives rise to a new hypothesis, in which NARD is not to be related to microangiopathy and instead shows a greater association with tubulointerstitial damage or macroangiopathy (i.e., arteriosclerosis).

Most diabetic patients with albuminuria show typical renal pathological changes, whereas typical diabetic glomerular changes are observed less frequently. In addition, atypical histologic changes suggestive of a severe interstitial or a tubular damage, or varying degrees of arteriosclerosis, were seen in patients with NARD [29]. Intrarenal arteriosclerosis is related to aging and hypertension. Furthermore, a recent study suggested that acute kidney injury (AKI) is a major component of CKD in patients with diabetes [30]. Both clinically evident and subclinical AKI can damage proximal tubular cells, podocytes, and endothelial cells, and such insults can create an apoptotic and inflammatory environment within the kidneys. Atubular glomeruli and glomerulotubular junction abnormalities in diabetes are also related to AKI and can lead to NARD [31].

2.4. Progressive renal decline (PRD)

Diabetic nephropathy has several phenotypes according to clinical and laboratory data; among them, PRD is the most serious. Generally, PRD is defined as a >3.5 mL/min/year loss in the eGFR in type 1 diabetes and PRD reasonably included NARD. Krolewski reported that the prevalence of PRD was 10, 32, and 50% in patients with normoalbuminuria, microalbuminuria, and macroalbuminuria, respectively [32]. The recent Scottish Go-DARTS study identified biomarkers for PRD: 154 patients with type 2 diabetes and CKD showed a >40% decline in eGFR during the 3.5-year study period [33]. In the second Joslin Kidney Study, in which PRD was defined as a decrease in eGFR >30% from baseline during ≤5 years of follow-up, an early decline in renal function developed in 6 and 18% of patients with normoalbuminuric and microalbuminuric diabetes, respectively [34]. Although the mechanism underlying such a decline is unclear, more intensive and personalized treatments are needed to prevent progression to ESRD.

The clinical course of diabetic nephropathy varies such that physicians should treat diabetic patients using tailored approaches; the term "natural course" may no longer be applicable in this era of active interventions. In future, more phenotype-specific approaches informed by gene- and proteome-based analyses are needed to improve patient prognosis.

3. Pathophysiology of diabetic nephropathy: tubule versus glomerulus

Glomerular dysfunction has long been considered a major driver of diabetic nephropathy. Kimmelstiel-Wilson nodules, which are characterized by the formation of diffuse nodular lesions of a pink hyaline material in glomerular capillary loops in the glomerulus [35], have contributed greatly to the identification of the glomerulus as the main culprit in the development of diabetic nephropathy. Diabetes-induced glomerulopathy can be caused by interactions among glomerular endothelial cells, mesangial cells, and podocytes via metabolic and hemodynamic perturbations [36]. However, glomerulopathy in diabetes is still not fully understood because various cells resident within the glomeruli have different roles in the disease process. Furthermore, recent studies revealed that glomerulopathy is preceded by tubular dysfunction during the development and progression of diabetic nephropathy [37]. These tubulocentric concept addressed in this chapter is summarized in **Figure 2**.
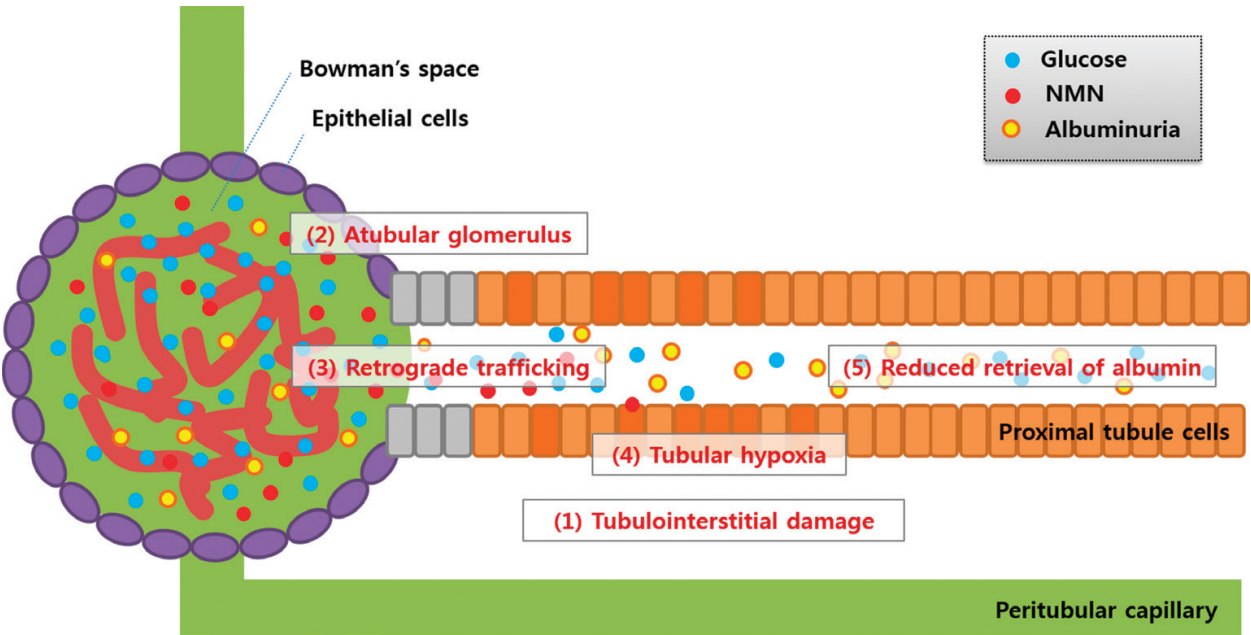

Figure 2. Tubulocentric concept for diabetic nephropathy. (1) Tubulointerstitial damage can cause a disconnect between glomerulus and tubule, (2) atubular glomerulus, (3) retrograde trafficking with NMN releasing by proximal tubule can contribute glomerulopathy, (4) proximal tubules are vulnerable to hypoxic injury, which can lead to fibrosis and apoptosis, (5) reduced retrieval of albumin by impaired tubule resorption is responsible for albuminuria in diabetic nephropathy.

3.1. Proximal tubules contribute to glomerulopathy

Pathological changes in the tubulointerstitium that have been linked to diabetic nephropathy include the thickening of the tubular basement membrane (TBM), tubular atrophy, interstitial fibrosis, and arteriosclerosis, which are closely correlated with the magnitude of renal dysfunction and albuminuria [38]. Furthermore, such tubulointerstitial damage can cause a disconnect between the glomerulus and the proximal tubule, the so-called atubular glomerulus, which is an important and a common cause of irreversible CKD progression [39, 40]. These glomerulotubular junction abnormalities accompanied by atubular glomerulus have been linked to the development and progression of diabetic nephropathy in both type 1 and 2 diabetes [31, 41]. Recent studies suggest that the glomerular dysfunction triggered by proximal tubules, the so-called retrograde trafficking might be important in diabetic nephropathy [42, 43]. Proximal tubules communicate with podocytes by releasing nicotinamide mononucleotide (NMN), and proximal tubule-specific Sirt1 protects against diabetic kidney disease by maintaining glomerular NMN concentrations and preserving podocyte function [42]. Furthermore, injured proximal tubule epithelium can trigger an inflammatory response, and repeated injury results in maladaptive repair. This in turn leads to tubulointerstitial fibrosis, tubular atrophy, and, potentially, secondary glomerulosclerosis, which is pathologically similar to classic diabetic nephropathy [44]. Albuminuria, which has been primarily considered as a glomerular damage marker, is a sensitive marker, reflecting the functional impairment in tubule, alone or in combination with glomerular origin in animal nephrotoxicity study [45]. Finally, a substantial evidence from human urinary biomarker data supports that proximal tubule damage might have an important role in the development of early diabetic nephropathy as a primary cause, not a secondary phenomenon [46].

3.2. Tubular hypoxia hypothesis

In diabetic kidney, proximal tubules are vulnerable to hypoxic injury because of an increased oxygen consumption, an impaired oxygen utilization, and a reduced oxygen delivery. Sodium reabsorption and gluconeogenesis processes occurring at the proximal tubules consume oxygen. The proximal tubule can be subdivided into three distinct segments (S1, S2, and S3) and is adapted for reabsorption. Transport across the tubular epithelium occurs via two routes: transcellular transport across luminal and basolateral membranes via Na^+, K^+ –adenosine triphosphatase (ATPase), and paracellular transport through tight junctions and the intercellular space. Glucose enters cells in the proximal tubule via the sodium-glucose cotransporter (SGLT), and is extruded from cells by GLUT1 and GLUT2 [47]. High Na^+, K^+-ATPase activity and oxygen consumption levels are needed to reabsorb glucose under high glucose conditions. A recent study showed that SGLT2 inhibitors downregulate Na^+ and K^+-ATPase activity and eventually reduce energy or oxygen requirements [48]. Similar to hepatocytes, epithelial cells in proximal tubules perform gluconeogenesis and export glucose into the circulation via oxygen- and energy-based processes. In diabetes, renal gluconeogenesis is particularly increased in the postprandial or fasting state [49]. Hypoxia induces apoptosis by upregulating Fas expression [50, 51]. Hypoxia stimulates extracellular matrix (ECM) expansion via transforming growth factor-β (TGF-β)-dependent and -independent pathways, such as an

increased collagen production, a decreased matrix metalloproteinase-2 (MMP-2) activity, and an increased tissue inhibitor of metalloproteinase-1 (TIMP-1) expression [52, 53].

Recently, our laboratory studied the role of MMP-2 in diabetic nephropathy. Hyperglycemia-induced oxidative stress is a major driver of diabetic nephropathy, and high glucose levels stimulated the induction of intracellular MMP-2 in HK2 cells; this expression was blocked by the NF-κB inhibitor pyrrolidine dithiocarbamate (PDTC) [54]. Intracellular MMP-2 exacerbates oxidative stress by inducing the mitochondrial permeability transition, which results in tubular epithelial cell-regulated necrosis [55]. Therefore, intracellular MMP-2 is related to oxidative stress, and proximal tubular cells are susceptible to hypoxic stress. This may be important in the pathogenesis of CKD in DM. The resultant may lead to glomerular change as well as tubulointerstitial hypoxia and finally loss of kidney function.

3.3. Intermittent and continuous injurious stimuli lead to proximal tubulopathy and CKD

Unlike healthy individuals, patients with diabetes are persistently exposed to various metabolic and hemodynamic factors that sustain the disease state [56]. In addition, AKI frequently occurs after various nephrotoxic insults, such as ischemia during cardiac surgery and those associated with the administration of contrast media. The proximal tubule is particularly vulnerable to the ischemia and toxin-mediated injury that lead to AKI. In a mouse model of induced proximal tubule injury, tubular regeneration after a single episode of renal epithelium injury was robust and efficient, leading to complete restoration of the kidney architecture [45]. However, repeated injury resulted in maladaptive repair, manifested as tubulointerstitial fibrosis and tubular atrophy, and with the potential for secondary glomerulosclerosis [45]. Thus, these data suggest that the cumulative effects of repeated episodes of subclinical AKI arising from injurious stimuli lead to the progressive tubulointerstitial fibrosis that is characteristic of CKD, including diabetic nephropathy. Epidemiological and clinical observations support a relationship between intermittent AKI and CKD progression in diabetic patients [57, 58]. AKI increased the risk of advanced CDK in diabetic patients independent of other major risk factors of kidney disease progression, and each episode of AKI showed a cumulative dose-response association, doubling the risk of stage 4 CKD [57]. In AKI, a low eGFR and/or an elevated albuminuria level are compelling biomarkers for major adverse outcomes and death in diabetes [58].

3.4. Tubular contribution to albuminuria

The role of proximal tubules in albuminuria in various renal disorders, including diabetic nephropathy, remains controversial. The glomerular filtration barrier has long been considered largely impermeable to albumin, but recent data suggest that it may not be especially important in this process [59]. According to the "retrieval hypothesis," albuminuria likely has a tubular origin, because albumin can be filtered by normal glomeruli in the nephrotic range if tubular reabsorption is only partial [60, 61]. Russo et al. [60] reported that more albumin was filtered and underwent a rapid retrieval process via transcytosis in proximal tubule cells. Therefore, controversy remains regarding the extent of the glomerular filtration of albumin. A study of Fanconi syndrome patients with proteinuria reported a markedly impaired albumin filtration rate [62]. Collectively, these data suggest that an increased glomerular leakage and

an impaired tubular reabsorption are not mutually incompatible, and both are accountable for albuminuria in the early diabetic nephropathy [61, 63].

4. Tubular biomarkers of diabetic nephropathy

Classification of diabetic nephropathy based on albuminuria and the eGFR provides prognostic information that is helpful to guide therapeutic decisions. Albuminuria serves as a marker of endothelial dysfunction, which is a prognostic factor for renal impairments and a high cardiovascular risk [64]. However, its progress is unpredictable, since microalbuminuria can regress toward normoalbuminuria, progress toward macroalbuminuria, or remain stable [65]. Moreover, diabetic nephropathy can develop in normoalbuminuric patients. In addition, structural changes in the glomerulus may appear before the onset of microalbuminuria, even though microalbuminuria is the established screening tool for diabetic nephropathy [66]. Therefore, an intensive search for new blood or urine biomarkers that could improve diagnostic and prognostic precision in diabetic nephropathy has recently been reported.

4.1. Classification

Because of emerging evidence supporting tubulocentric concepts in diabetic nephropathy, the focus has shifted from glomeruli to proximal tubules, which may contribute to the pathogenesis of diabetic nephropathy from an early stage. Both functional and structural markers can be used to detect proximal tubule dysfunction in diabetic nephropathy. One method is to detect filtered proteins due to the impaired reabsorption by the proximal tubules. The main site for reabsorbing filtered proteins is the proximal tubules and, assuming no secretion or degradation of these proteins through the glomerulus, the more proteins are filtered, the higher the urinary excretion rate will be when tubular reabsorption is destroyed. These functional tubular biomarkers are low-molecular weight proteins (LMWP) that are mostly reabsorbed by the proximal tubules. Another method is to detect proteins released into the urine by tubular injury. These urinary proteins are structural tubular biomarkers that come directly from tubular cells rather than from plasma. The principal tubular biomarkers in diabetic nephropathy are briefly described in **Table 1**.

4.2. Clinical utility

In tubular proteinuria, the endocytic function of proximal tubule is damaged and a large amount of LMWP is detected in the urine. For example, retinol-binding protein 4 is markedly elevated when endocytic function is completely eliminated [67]. The cause of an increased LMWP excretion in diabetes is usually explained by tubular disease. Animal models suggest the pathway that the filtered proteins compete with each other for reabsorption in proximal tubules [68]. Clinical studies have also shown that the same pathway leads to the reabsorption of albumin and LMWP through glomeruli [69]. The ability of protein reabsorption in proximal tubules is not known but competition for reabsorption between albumin and LMWP may occur. As a result, a slight increase in filtered albumin through glomeruli in the early stage of diabetic nephropathy will not cause albuminuria, but an increase in LMWP excretion may be detected indirectly. In other words, early glomerular injury in diabetes may not

Functional tubular biomarkers		
Albumin	65 kDa	Normally filtered very little at the glomerulus. With glomerular barrier damage, filtration occurs and followed by tubular reabsorption. The resulting albuminuria reflects the combined contribution of these two processes.
Cystatin C	13 kDa	Filtered by the glomerulus and reabsorbed in the proximal tubule. No tubular secretion.
Retinol-binding protein 4	21 kDa	
α_1-microglobulin	26–31 kDa	
β_2-microglobulin	11.8 kDa	Filtered by the glomerulus and degraded in the proximal tubule via a megalin-dependent pathway. Unstable in urine.
Structural tubular biomarkers		
Neutrophil gelatinase-associated lipocalin (NGAL)	25 kDa	Hyper-produced in the kidney tubules within a few hours after renal ischemia-reperfusion injury. It is freely filtered and reabsorbed in the proximal tubule.
Kidney injury molecule-1 (KIM-1)	70–80 kDa	Cleaved and released into the lumen of the tubule. It facilitates repair of the damage by removing cellular debris and apoptotic bodies from the injured tubulointerstitial compartment.
N-acetyl-β-D glucosaminidase (NAG)	>130 kDa	Plasma NAG is not filtered through the glomeruli. It is released into the urine after renal tubule injury.
Liver-type fatty acid-binding protein (L-FABP)	14.2 kDa	Associated with structural and functional tubular damage. It is freely filtered and reabsorbed in the proximal tubule.
Megalin and Cubilin	Megalin 600 kDa Cubilin 460 kDa	Most proteins filtered through glomeruli have been identified as ligands of megalin, cubilin, or both. The central mechanism for protein reabsorption in the proximal tubule.
Alkaline phosphatase (ALP) and γ-glutamyltransferase (GGT)	ALP 70–120 kDa GGT 90 kDa	ALP originates from damaged renal tubules, and its levels are associated with the degree of damage. Increased GGT excretion in the urine reflects the damage of the brush-border membrane and the loss of microvilli.

Table 1. Principal functional and structural tubular biomarkers overexpressed in the urine and explored in clinical background of diabetic nephropathy [65–67].

cause albuminuria if proximal tubules are functioning normally and can reabsorb the excess albumin filtered from glomerulus. Other clinical study has also suggested the dissociation between albuminuria and increased glomerular leakage of albumin [70].

4.3. Predictive value in clinical studies

Studies using tubular biomarkers showed conflicting results regarding their predictive value for GFR decline or the development of albuminuria. In a retrospective analysis, two tubular injury biomarkers, β2 microglobulin and N-acetyl-β-D glucosaminidase (NAG), did not show prognostic utility for detecting GFR decline in type 2 DM (T2DM). However, histologic findings of interstitial fibrosis and tubular atrophy (IFTA) did have prognostic benefit. Both

$\beta2$ microglobulin and NAG showed a statistically significant correlation with IFTA scores, identified as an independent predictor of progression to diabetic nephropathy [71]. In a nested case-control study from the diabetes control and complications trial (DCCT), both the baseline NAG and increase NAG over time predicted albuminuria independently [72]. A 3-year prospective study found that type 1 DM (T1DM) patients with high levels of urinary neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) had a rapid deterioration in GFR. This suggests that tubular injury is important for the progression of diabetic nephropathy [73]. Fu et al. [74] showed that NGAL increased significantly from healthy controls to normoalbuminuric, microalbuminuric, and macroalbuminuric patients with T2DM. Conway et al. [75] revealed that the uKIM-1/Cr ratio was elevated in T2DM patients with early-stage nephropathy, suggesting tubular injury. The uKIM-1/Cr ratio was correlated with a rapid decline in GFR and the severity of proteinuria. Soggiu et al. [76]showed that increased RBP4 and $\alpha1$-microglobulin excretion could predict early-stage nephropathy in T1DM. In a retrospective cohort study of 1549 patients with T1DM, liver-type fatty acid-binding protein (L-FABP) was a valuable predictor of the progression of diabetic nephropathy, irrespective of disease stage [77]. Our laboratory reported that albuminuria is significantly correlated with three tubular biomarkers (KIM-1, NGAL, and L-FABP) during the early stage of diabetic nephropathy [78]. Our laboratory also reported results obtained from 237 patients with T2DM who were measured for NAP and cystatin C. Both biomarkers were significantly associated with the decline in eGFR after adjusting for clinical parameters [23]. Prospective studies are needed to confirm the clinical utility of tubular biomarkers in the early stage of diabetic nephropathy.

4.4. Proteomics and microRNA approach

Recently, many researches using high-throughput proteomics and microRNA (miRNA) approaches have been introduced in the field of diabetic nephropathy. These two novel approaches for discovering biomarkers can be used to explore diabetic nephropathy through multiple pathophysiological processes that can reflect complexed structural and functional pathways. Proteomics might provide dynamic profiles, reflecting the complexed pathophysiological changes that occur at different stages of diabetic nephropathy. Proteomics could serve as early biomarkers (e.g., CKD273 classifier, a panel consisting of 273 urinary peptides [79]) with a good predictive value in the clinical environments [80]. However, proteomic and miRNA approaches have yet not been able to replace albuminuria as a marker of diabetic nephropathy. miRNAs, which are small noncoding RNAs, are found in extracellular environment including various body fluids and function in posttranscriptional regulation of gene expression. The majority of miRNAs are located within the cell and can serve as a potential biomarker. In the urine, miRNAs are more stable in degradation than proteins and are valuable for urinary biomarkers. If miRNAs are handled and stored carefully, it could promote the discovery of novel urinary biomarkers for diabetic nephropathy. However, they were differentially expressed in T1DM and T2DM, and differed according to miRNA sources. In addition, miRNAs were reported to show gender-specific differences in T1DM [81]. Therefore, further studies are needed to optimize the utility of miRNAs in clinical practice.

5. Proximal tubules as therapeutic targets

Chronic hyperglycemia is an essential component of diabetes and the principal risk factor for microvascular complications, including diabetic nephropathy [82]. Patients with diabetes also have other risk factors such as obesity, systemic hypertension, and dyslipidemia. Despite advances in pharmacologic interventions (e.g., RAS blockers) to control these risk factors, the prevalence of diabetic nephropathy continues to rise and remains the leading cause of ESRD worldwide [83]. Several novel therapeutic strategies, including dual/triple RAS blockade and sulodexide and bardoxolone therapy, have been sought to improve renal outcome in diabetes [83]. However, these approaches proved either ineffective or harmful, suggesting that other strategies should be sought [84]. The optimal prevention and treatment of CKD in patients with diabetes requires the implementation of therapies that specifically consider the role played by proximal tubules [85]. The dimension and function of proximal tubules increase in response to a higher glucose load. These changes have been linked to an increase in GFR, or the so-called diabetic hyperfiltration [85]. Thus, considering the importance of proximal tubules in diabetic nephropathy, the development of novel antidiabetic agents, such as SGLT 2 inhibitors, could yield new tools to prevent diabetic nephropathy.

5.1. Glucose handling by the kidney

The glomeruli of normoglycemic healthy individuals filter ~140–160 g of glucose each day. This would result in a urinary loss of energy substrate equal to ~30% of the daily energy expenditure if not reclaimed by the renal tubules. Two glucose transporters are responsible for renal glucose reabsorption, SGLT1 and SGLT2, which are secondary active co-transporters located on the apical membrane that couple glucose reabsorption to sodium reabsorption. SGLT2 is located in the early (S1) proximal tubule and accounts for 90% of glucose reabsorption, while SGLT1 is located in the more distal part of the proximal tubule (S2/S3) and accounts for the remaining 10% [86].

5.2. Glucose reabsorption in the diabetic kidney

In diabetes, hyperglycemia is maintained by the alterations in kidney. Both renal gluconeogenesis and glucose reabsorption are increased in diabetic subjects [3]. Hyperglycemia increases the amount of glucose filtered through the kidney, and the maximum capacity of resorption for glucose is increased by ~30% to ~500–600 g/day in patients with type 2 diabetes [84]. If the filtered glucose load exceeds the threshold of proximal tubules in diabetes, glucosuria increases in a linear fashion. These latter changes occur in parallel with upregulated SGLT2 expression [87]. More specifically, an increased capacity for glucose transport may contribute to the enhanced renal glucose reabsorption seen in diabetes. Upregulated renal SGLT2 levels have been reported in both human cells and some animal models of type 1 and type 2 diabetes [88]. Proximal tubule growth (hypertrophy) is a key feature of early-stage diabetes, which may explain the increased capacity for renal glucose reabsorption [61]. However, it remains unclear whether SGLT2 upregulation is the result of proximal tubule hypertrophy in diabetes.

5.3. SGLT2 inhibition as a therapeutic target

Based on the observation that SGLT2 has an important role in renal glucose reabsorption, the proximal tubules of the kidney have been targeted to control the blood glucose. SGLT2 inhibitors are a novel class of antidiabetic drugs that recently entered the market. These medications target the kidney proximal tubules to block glucose reabsorption, thereby inducing urinary glucose excretion and reducing circulating plasma glucose levels. Their mechanisms of action are independent of the action of insulin and beta-cell function.

Phlorizin promotes glucosuria and lowers serum glucose levels in diabetic patients, and completely inhibits renal glucose reabsorption in humans [89]. However, the clinical use of phlorizin was not pursued due to poor intestinal absorption, low bioavailability, and lack of selectivity for SGLT2. Additionally, phlorizin is hydrolyzed to phloretin in the gut, which inhibits multiple GLUTs [90]. Thus, the development of SGLT2-specific inhibitor was an important breakthrough for therapies targeting renal glucose transport for blood glucose management [91].

Currently, the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have approved three oral SGLT2 inhibitors (canagliflozin, dapagliflozin, and empagliflozin) for patients with type 2 diabetes. The SGLT2 inhibitors reduced HbA1C by 0.5–0.7% [92]. Additional drugs within this class are under development (**Table 2**). The glucose-lowering effect of SGLT2 inhibitors is closely related to the amount of filtered glucose. Since SGLT2 is responsible for >90% of glucose reabsorption by the kidney, its inhibition would be expected to induce a urinary glucose loss close to the filtered load (160–180 g/day in normoglycemia). However, SGLT2 inhibitor-associated urinary glucose excretion is only ~40–80 g/day in healthy individuals and patients with type 2 diabetes, suggesting that SGLT1

Generic name (trade name)	Company	Dosing	SGLT2/SGLT1 selectivity
Dapagliflozin (Forxiga/Farxiga)	AstraZeneca	5–10 mg QD	1400
Canagliflozin (Invokana)	Janssen	100–300 mg QD	160
Empagliflozin (Jardiance)	Boehringer Ingelheim	10–25 mg QD	5000
Ipragliflozin (Suglat)	Astellas Pharma	25–100 mg QD	570
Tofogliflozin (Apleway/Deberza)	Sanofi/Kowa	20 mg QD	1875
Luseogliflozin (Lusefi)	Taisho Pharmaceutical	2.5–5 mg QD	1770
Ertugliflozin (Steglatro)	Merck/Pfizer	1–25 mg QD	2200
Sotagliflozin[a] (N.A.)	Lexicon Pharmaceuticals	400 mg QD	20
Remogliflozin etabonate (N.A.)	BHV Pharma	100–400 mg QD	1100
Henagliflozin (N.A.)	Jiangsu HengRui Medicine	2.5–200 mg QD	1800

SGLT2/SGLT1, sodium-glucose cotransporter 2/sodium-glucose cotransporter 1; QD, once daily; N.A., not applicable.
[a]Dual SGLT1/2 inhibitor.

Table 2. SGLT2 inhibitors currently approved or in development.

has an important role in glucose reabsorption under SGLT2 inhibition [88]. SGLT2 inhibitor decreases insulin levels and increases glucagon levels. Thus, SGLT2 inhibitor enhances endogenous glucose production, thereby reducing the glucose-lowering efficacy [93].

5.3.1. Effects of SGLT2 inhibitors on renal and cardiovascular outcomes

The EMPA-REG OUTCOME and CANVAS trials investigated the effects of empagliflozin and canagliflozin on renal and cardiovascular outcomes in type 2 diabetes patient with high cardiovascular risk factors and an eGFR of ≥30 mL/min/1.73 m^2. In the EMPA-REG OUTCOME trial, empagliflozin was associated with a relative risk reduction of 39% in incident or worsening nephropathy (progression to macroalbuminuria, doubling of serum creatinine level, initiation of renal replacement therapy, or death from renal disease). Moreover, empagliflozin was associated with significant risk reductions of 44 and 55% in doubling serum creatinine and the initiation of renal replacement therapy, respectively [94]. Empagliflozin was also associated with relative risk reductions of 38, 35, and 32% in cardiovascular death, hospitalization for heart failure, and death from any cause, respectively. However, there was no risk reduction in nonfatal myocardial infarction or in nonfatal stroke [95]. In the CANVAS trial, canagliflozin was associated with relative risk reductions of 27 and 40% in the risk of albuminuria progression and composite renal outcome (40% reduction in eGFR, the need for renal replacement therapy, or death from renal cause), respectively [96]. Canagliflozin was also associated with a relative risk reduction of 14% in primary cardiovascular composite outcome (cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke) [96].

5.3.2. SGLT2 inhibitors: beyond glucose lowering

The beneficial effects of SGLT2 inhibitors could be associated with a glucose-lowering effect. However, the small HbA1C reduction is unlikely to explain the rapid onset and effect size. Therefore, pleiotropic effects of SGLT2 inhibitor likely played a role (**Figure 3**). A meta-analysis of randomized controlled trial demonstrated that SGLT2 inhibitors decrease the systolic blood pressure by 3–6 mmHg in type 2 diabetes patients [92]. The blood pressure-lowering effect of SGLT2 inhibitors is partly associated with glycosuria-accompanied osmotic diuresis, which increases urine output by 200–600 mL/day. SGLT2 inhibitors also induce natriuresis by decreasing sodium reabsorption in the proximal tubules [84]. Additionally, a positive interaction between SGLTs and Na$^+$/H$^+$ exchanger-3 (NHE3), and the inhibition of NHE3 with phlorizin at sites associated with a reduced NHE3 activity have been described [97]. Since NHE3 in the early proximal tubule is responsible for up to 30% of fractional sodium reabsorption, its potentially downregulated activity on SGLT2 inhibition may contribute to natriuresis and subsequent GFR and blood pressure lowering; however, this hypothesis requires further study [98].

Clinical trials with SGLT2 inhibitors in patients with type 2 diabetes showed a significant weight reduction of ~1.7 kg or 2.4% compared with placebo [99]. While initial weight loss appears to result from SGLT2 inhibitor-associated osmotic diuresis, steady-state weight loss with SGLT2 inhibitor is thought to be associated with a reduction in body fat mass. In obese rat, SGLT2 inhibitor reduces fat mass with a steady calorie loss by increasing lipolysis and fatty acid oxidation. SGLT2 inhibitor-induced fat loss is also associated with the increased
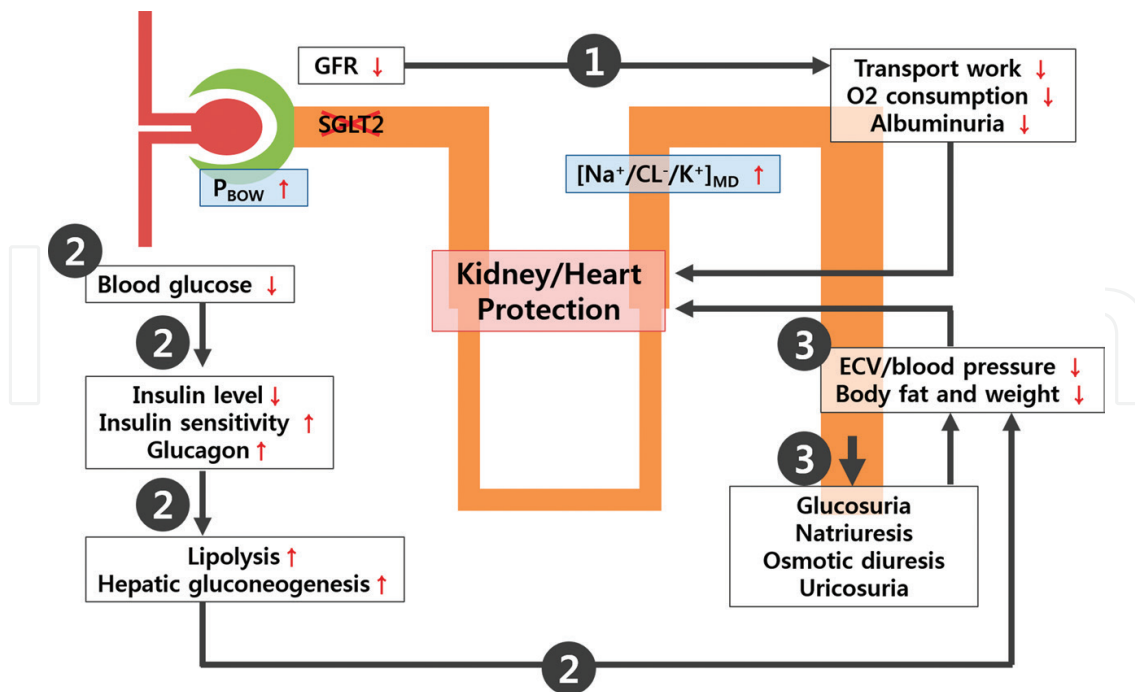
Figure 3. Pleiotropic effects of sodium-glucose cotransporter (SGLT2) inhibitor. SGLT2 inhibitor may have beneficial effects on kidney and heart via several pleiotropic mechanisms: (1) SGLT2 inhibitor blocks glucose hyper-reabsorption in the proximal tubule of the diabetic kidney, increasing the tubuloglomerular feedback signal at the macula densa ($[Na^+/Cl^-/K^+]_{MD}$) and hydrostatic pressure in Bowman's space (P_{BOW}).This reduces albuminuria and tubular transport work and, thus, renal oxygen consumption by decreasing glomerular hyperfiltration. (2) SGLT2 inhibition reduces insulin levels and increases insulin sensitivity and glucagon levels. As a consequence, lipolysis and hepatic gluconeogenesis are elevated. These metabolic adaptations reduce fat tissue and body weight. (3) SGLT2 inhibitors induce a modest osmotic diuresis, natriuresis, glucosuria, and uricosuria, which can reduce extracellular volume (ECV), blood pressure, serum uric acid levels, and body weight.

use of fatty acids instead of glucose as an energy source [100]. SGLT2 inhibitor has also been reported to reduce the body weight by reducing visceral and subcutaneous adipose tissue in type 2 diabetes patients [101].

Serum uric acid-lowering effect of SGLT2 inhibitor may be associated with the improved renal and cardiovascular outcome. SGLT2 inhibitor induces glycosuria, thereby facilitating intracellular uric acid exchange via GLUT9 isoform 2 at the proximal tubule, thereby enhancing urinary excretion of uric acid [102]. However, further study is mandatory to verify the precise mechanism of uricosuric effect of SGLT2 inhibitor.

Glomerular hyperfiltration is a detrimental process in diabetic nephropathy and increases intraglomerular pressure. The complicated interaction of hyperglycemia-induced structural and hemodynamic alterations causes the glomerular hyperfiltration [84]. By inducing baro-trauma and shear stress, it exacerbates albuminuria and likely contributes to the development and progression of CKD [84]. SGLT2 inhibition attenuates primary tubule hyper-reabsorption in diabetes and thereby reduces glomerular hyperfiltration. Specifically, SGLT2 inhibitors increase sodium delivery at the macula densa and subsequently activate tubuloglomerular feedback, which induce afferent arteriolar vasoconstriction and then reduce intraglomerular pressure [84]. Recent studies have also confirmed that the SGLT2 inhibitors lower GFR. The

empagliflozin decreased the eGFR by 19% in type 1 diabetes patients. The canagliflozin also initially decreased GFR in patients with type 2 diabetes [103]. After an initial decrease in eGFR, canagliflozin-treated group showed the slower decline of eGFR compared with glimepiride-treated group over 2 years independently of glycemic effects [104].

5.3.3. SGLT2 inhibitors in diabetic CKD

Nephrons that survive in the advanced stages of CKD are assumed to hyper-filter as a way of compensating for the loss of other nephrons. In the short term, SGLT2 inhibitors decreased the eGFR in patients with type 2 diabetes and stage 2 or 3 CKD [105, 106]. In the long term, the amelioration of glomerular hyperfiltration by SGLT2 inhibitor in CKD may preserve the integrity of the remaining nephrons. This concept has also been suggested for angiotensin II inhibition. Indeed, both SGLT2 and angiotensin II inhibition confer additional renoprotective effect in type 2 diabetes patients with basal eGFR of >30 mL/min/1.73 m^2 [94].

5.4. SGLT2 inhibitors: future perspectives

The kidney and cardiovascular protection is likely to be attributed to the pleiotropic effects of SGLT2 (EMPA-REG OUTCOME and CANVAS trials). Future research is required to assess their ability to improve renal outcome in diabetic patients with more advanced CKD. The large trials with different SGLT2 inhibitors are ongoing to confirm whether their beneficial effects are drug-specific or represent a class effect. It is also important to investigate their effect in patients with nondiabetic kidney disease. In addition, dual SGLT1/2 inhibitors are under development to maximize the beneficial effect of SGLT2 inhibitors without causing side effects associated with SGLT1 inhibitors.

6. Conclusions

Although researchers are trying to determine the pathophysiology of diabetic nephropathy, our understanding remains incomplete. A recent paradigm shift to a tubulocentric concept for diabetic nephropathy implies that the proximal tubules have a central role in the disease process, rather than being secondarily affected by other components during the development of diabetic nephropathy. Representing a considerable step toward shifting the glomerulotubular balance, these new perspectives might lead to significant diagnostic and therapeutic advances in diabetic nephropathy.

Acknowledgements

Disclosures

The authors have nothing to disclose.

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