Pathophysiology of diabetic kidney disease: impact of SGLT2 inhibitors

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Abstract | Diabetic kidney disease is the leading cause of kidney failure worldwide; in the USA, it accounts for over 50% of individuals entering dialysis or transplant programmes. Unlike other complications of diabetes, the prevalence of diabetic kidney disease has failed to decline over the past 30 years. Hyperglycaemia is the primary aetiological factor responsible for the development of diabetic kidney disease. Once hyperglycaemia becomes established, multiple pathophysiological disturbances, including hypertension, altered tubuloglomerular feedback, renal hypoxia, lipotoxicity, podocyte injury, inflammation, mitochondrial dysfunction, impaired autophagy and increased activity of the sodium–hydrogen exchanger, contribute to progressive glomerular sclerosis and the decline in glomerular filtration rate. The quantitative contribution of each of these abnormalities to the progression of diabetic kidney disease, as well as their role in type 1 and type 2 diabetes mellitus, remains to be determined. Sodium–glucose co-transporter 2 (SGLT2) inhibitors have a beneficial impact on many of these pathophysiological abnormalities; however, as several pathophysiological disturbances contribute to the onset and progression of diabetic kidney disease, multiple agents used in combination will likely be required to slow the progression of disease effectively.

Diabetes mellitus is a global epidemic. In the USA, its prevalence has increased progressively over the past 20 years and is now estimated to affect 10.5% of the US population or 34.2 million individuals[1]. Much of the morbidity and mortality of diabetes relates to the development of complications that affect organ systems such as the kidney. Diabetic kidney disease (DKD) is a chronic progressive disorder that can lead to kidney failure and is currently the leading cause of kidney replacement therapy — both dialysis and transplantation — in the USA and worldwide[2]. In contrast to other diabetic complications, the prevalence of DKD has not changed significantly over the past 30 years[3]. Current therapies for DKD, including blood pressure control with angiotensin-converting enzyme (ACE) inhibitors or angiotensin-receptor blockers (ARBs) and strict glycaemic control, have been modestly successful in delaying the onset and/or progression of kidney damage[4]. However, renoprotective effects of a new class of antidiabetic agent — sodium–glucose cotransporter 2 (SGLT2) inhibitors — have now been demonstrated. Cardiovascular outcome trials with SGLT2 inhibitors demonstrated the ability of these drugs to reduce composite renal outcomes (doubling of serum creatinine, development of macroalbuminuria, need for dialysis and/or transplantation or kidney death) by 40–70%[5–7] in patients with type 2 diabetes mellitus (T2DM). Positive outcomes from the CREDENCE trial[8] led to FDA approval of canagliflozin for the treatment of DKD in 2019. Most recently, the DAPA-CKD trial, which included patients with chronic kidney disease (CKD) both with and without T2DM[9], demonstrated that dapagliflozin reduced the composite kidney end point by 31% (HR 0.61, 95% CI 0.51–0.72, $P < 0.0001$).

Early alterations in the kidneys of patients with both type 1 and type 2 diabetes include the development of glomerular hyperfiltration and hypertrophy, followed by thickening of the glomerular basement membrane, mesangial matrix accumulation, the development of nodular glomerulosclerosis, increased urinary albumin excretion (UAE) and ultimately progression to kidney failure[10,11]. Identification of the mechanisms underlying these processes is necessary to understand the renoprotective effects of SGLT2 inhibitors and/or for the development of future therapies[4].

This Review focuses on the multifactorial pathophysiological mechanisms that underlie the development of DKD. We describe the mechanisms by which SGLT2 inhibitors modify many, but not all, of these pathophysiological disturbances and discuss how these actions might contribute to their renoprotective effects. This perspective emphasizes that no single factor can explain the development of DKD and that drug combinations will likely be required to slow or prevent the development

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Key points

- Multiple pathophysiological disturbances contribute to the onset and progression of diabetic kidney disease (DKD); from a clinical standpoint, this multifactorial pathogenic process implies that the use of multiple agents in combination will be required to treat the disease.
- Hyperglycaemia and hypertension are the key factors responsible for the development of DKD, but, once established, even tight glycaemic and/or hypertension control may not be able to halt or slow disease progression.
- Other pathogenic processes underlying the development and progression of DKD include alterations in tubuloglomerular feedback, tubule hypertrophy, hypoxia, podocyte injury, albuminuria and lipotoxicity.
- Inflammation, endothelial dysfunction, mitochondrial injury, fibrosis and impaired autophagy also contribute to the progressive nature of DKD, but these defects are most likely to be secondary events that follow the primary events described above. Sodium–glucose co-transporter 2 (SGLT2) inhibitors correct or improve many of the pathological processes involved in the development of and progression of DKD and are likely to underlie the ability of these agents to slow progression of established DKD in large prospective clinical trials; of these mechanisms, restoration of normal tubuloglomerular feedback and reduced interglomerular pressure are especially pertinent.

of DKD in patients with diabetes. The contribution of genetics and epigenetics to DKD has been covered elsewhere and is not discussed in this Review.

Pathogenesis of diabetic kidney disease

The pathogenesis of DKD is multifactorial with many diverse structural, physiological, haemodynamic and inflammatory processes contributing to the progressive decline in glomerular filtration rate (GFR) (FIG. 1). Increased activity of the SGLT2 transporter, which is responsible for ~90% of glucose reabsorption and the majority of sodium reabsorption in the proximal tubule, has a central role in initiating many of these pathophysiological abnormalities. Conversely, inhibition of SGLT2 reverses many of these disturbances and markedly slows the progression of DKD.

Hyperglycaemia

Hyperglycaemia is the predominant factor underlying the development of DKD (FIG. 2). Patients with HbA_{1c} within the normal range (<5.7%) do not develop DKD, whereas microalbuminuria occurs in 15–20% of individuals with 'prediabetes' (HbA_{1c} 5.7–6.4%)[12]. However, the proportion of individuals with microalbuminuria who progress to macroalbuminuria and eventually to CKD while HbA_{1c} remains in the prediabetic range is unknown. In the Diabetes Control and Complications Trial[13], 43% of patients with type 1 diabetes mellitus (T1DM) and microalbuminuria progressed to macroalbuminuria over 10 years, but none experienced a decline in GFR. Primary strategies to prevent the development of DKD should be aimed at maintaining HbA_{1c} <6.5%. When HbA_{1c} exceeds 7.0%, the incidence of microalbuminuria begins to rise steeply and progressively over time[14].

Of note, intensive glycaemic control in the Diabetes Control and Complications Trial[13] reduced the incidence of albuminuria by 50% and this beneficial effect persisted for more than a decade after completion of the trial[15,16]. Similarly, the United Kingdom Prospective Diabetes Study in patients with T2DM[17] demonstrated that each ~1% reduction in HbA_{1c} reduced the risk of microvascular complications by 37%; specifically, the risk of microalbuminuria was decreased by 33%. The Action in Diabetes and Vascular Disease[18] and the Action to Control Cardiovascular Risk in Diabetes[19] trials also showed significantly lower rates of albuminuria with intensive glycaemic control, although not in patients with T2DM and advanced nephropathy[19]. A major unanswered question is at what level of albuminuria (and GFR) does intensive glycaemic control fail to slow the progression of established DKD. Although normalization of the mean blood glucose level may slow progression of DKD in individuals with high levels of albuminuria[20,21], it is unlikely to completely halt progression[22,23]. Also of note is the fact that ~20% of patients with diabetes develop DKD in the absence of albuminuria[24]. Thus, methods other than albuminuria, including assessment of kidney histology on biopsy, are needed to identify patients with diabetes who are at risk of DKD development and progression.

SGLT2 inhibitors and hyperglycaemia. SGLT2 inhibitors reduce both fasting and postprandial plasma glucose concentrations[25] and, in individuals with T2DM and HbA_{1c} 8.0–8.5%, they decrease HbA_{1c} by ~1.0%[26]. Mechanistically, SGLT2 inhibitors work by reducing the renal threshold for glucose excretion from ~10 mmol/l (180 mg/dl) to ~2.2 mmol/l (~40 mg/dl)[27]. The resultant glucosuria decreases the mean plasma glucose concentration and ameliorates glucotoxicity, resulting in improved β-cell function and enhanced insulin sensitivity[25,27–29]. Unlike muscle, adipocytes and hepatocytes, in which glucose uptake is mediated by insulin-stimulated GLUT4 activity, glucose uptake by kidney cells is not insulin-regulated and increases in proportion to the plasma glucose concentration. In the context of diabetes mellitus, this unregulated glucose uptake floods cells of the kidney glomerulus and tubules with glucose and diverts glucose into non-glycolytic pathways including the hexosamine and aldose reductase pathways, resulting in the glycosylation of proteins and generation of advanced glycation end products, which promote mitochondrial dysfunction, oxidative stress and inflammation[30] (FIG. 2). By reducing plasma glucose levels, SGLT2 inhibitors reverse these adverse intracellular metabolic effects.

Hypertension

After hyperglycaemia, hypertension is the next most important factor that contributes to the progression of DKD[31,32]. Normotensive patients with advanced DKD show slower progression of kidney disease than do patients with hypertension[33]. However, the 'optimal' blood pressure target for patients with DKD has long been a subject of debate. The American Diabetes Association and Joint National Committee 8 recommend blood pressure <140/90 mmHg for patients with diabetes and <130/80 for individuals at high risk of cardiovascular disease, whereas the American Heart Association and American College of Cardiology joint 2017 guidelines recommend the lower target of <130/80 mmHg for all individuals with diabetes and CKD. In the Action to Control Cardiovascular Risk in Diabetes study, targeting

Angiotensin escape
The inability of angiotensin-converting enzyme inhibitors and angiotensin-receptor blockers to reduce circulating angiotensin II concentrations to undetectable levels.

Aldosterone escape
The inability of angiotensin-converting enzyme inhibitors and angiotensin-receptor blockers to reduce circulating aldosterone concentrations to undetectable levels.

a systolic blood pressure target <120 mmHg failed to reduce cardiovascular events in patients with T2DM and was associated with increased rates of hyperkalaemia and elevations in serum creatinine level[34]. By contrast, SPRINT showed a benefit of targeting lower blood pressure (systolic blood pressure <120 mmHg) but recruited mainly non-diabetic elderly patients[35]. Thus, the optimal blood pressure target to maximally slow or prevent progression of DKD in patients with established kidney disease is unknown.

Activation of the renin–angiotensin–aldosterone system (RAAS) has an important role in the pathophysiology of DKD, and the renoprotective effect of RAAS blockade with ACE inhibitors or ARBs is well established[36,37]. The renoprotective effect of RAAS blockers extends beyond their effects on systemic blood pressure, as they also reduce the elevated intraglomerular pressure that is characteristic of DKD[4,11,36,37]. However, RAAS blockers do not completely abrogate the progression of DKD, possibly as a result of angiotensin escape and/or aldosterone escape[38], leading to increased renin activity following prolonged RAAS inhibition. Of note, however, the renin inhibitor aliskiren failed to produce any added beneficial effect on prevention of DKD above that afforded by RAAS blockade[39], suggesting that mechanisms other than angiotensin escape and/or aldosterone escape underlie the incomplete response of DKD to RAAS blockade.

Of note, the mineralocorticoid receptor antagonists, spironolactone and eplerenone, reduce proteinuria in patients with DKD when administered alone and produce additional antiproteinuric benefits when added to ACE inhibitors or ARBs[40,41] This additive antiproteinuric benefit is independent of blood pressure reduction[40,41], suggesting that other mechanisms including inhibition of inflammation and fibrosis contribute to their renal protective effect.

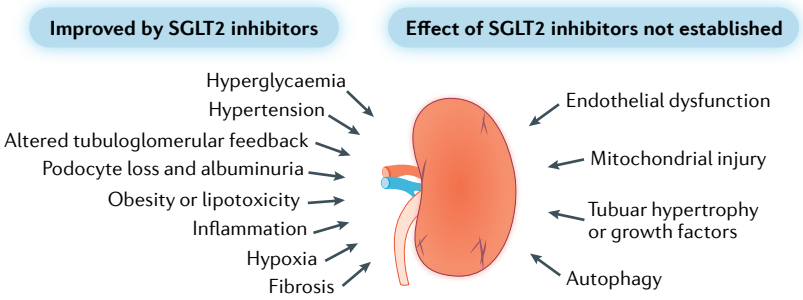

Fig. 1 | **The pathophysiology of diabetic kidney disease.** In the absence of hyperglycaemia, diabetic kidney disease (DKD) does not occur. However, once hyperglycaemia is established, multiple pathophysiological disturbances contribute to the progression of kidney damage and decline in glomerular filtration rate. Hypertension is especially damaging. Although multiple other mechanisms contribute to the decline in kidney function and structural damage, the weighted contribution of each individual factor remains to be elucidated. Primary prevention should be aimed at normalizing blood glucose levels. In addition to reducing hyperglycaemia, sodium–glucose co-transporter 2 (SGLT2) inhibitors affect a number of other pathogenic pathways that underlie DKD. However, the involvement of multiple pathophysiological disturbances suggests that once DKD is established, multiple agents may be required to reverse the pathogenic abnormalities.

SGLT2 inhibitors and hypertension. A growing body of evidence demonstrates that SGLT2 inhibitors are equally as, if not more, effective in preventing the progression of DKD as ACE inhibitors or ARBs and that their effect is additive to that of RAAS blockers[5–8]. SGLT2 inhibitors induce a negative sodium and water balance of ~100 mEq and ~700 ml, respectively, within the first 48–72 h of their administration[25] and this decrease in intravascular volume is associated with a 4–5/1–2 mmHg decline in blood pressure[26]. Consistent with this mild intravascular volume depletion, activation of the RAAS system occurs with use of SGLT2 inhibitors[42], excluding a reduction in angiotensin levels as a cause of the decrease in blood pressure. Although the drop in blood pressure with SGLT2 inhibition could contribute to their renoprotective effect, the decrease is modest and other factors are likely to dominate[43]. Inhibition of sympathetic nervous system activity has been suggested as a cause of blood pressure reduction with SGLT2 inhibitor use, but this effect remains to established.

Altered tubuloglomerular feedback

A series of elegant studies performed over 30 years ago[44] demonstrated the important role of glomerular capillary hypertension in the progression of kidney disease. Sodium chloride delivery to the macula densa cells of the juxtaglomerular apparatus (JGA) has a central role in the regulation of GFR and intraglomerular pressure through the phenomenon of tubuloglomerular feedback[44,45]. Specifically, a reduction in the delivery of sodium chloride to the macula densa increases GFR and intraglomerular pressure. By contrast, increased delivery of sodium chloride to the macula densa reduces GFR and intraglomerular pressure through tubuloglomerular feedback. The reduction in GFR and intraglomerular pressure involves the release of ATP, which induces the subsequent formation of adenosine and activation of adenosine receptors in the afferent arterioles to promote afferent vasoconstriction while, concurrently, local inhibition of the RAAS causes efferent vasodilation[46,47] (FIG. 3).

Filtered glucose is reabsorbed in the proximal tubule by two sodium-dependent glucose transporters, SGLT1 and SGLT2, with SGLT2 responsible for the bulk (~90%) of glucose absorption[48]. In patients or animal models with poorly controlled diabetes, the increased filtered load of glucose leads to an increase in sodium-coupled glucose reabsorption by the proximal tubule and decreased sodium delivery to the macula densa[49,50]. This decrease in sodium delivery to the JGA results in intrarenal activation of the RAAS, efferent arteriolar vasoconstriction, glomerular hypertension and kidney hyperfiltration[44,45,50,51]. Decreased delivery of sodium to the macula densa also inhibits ATP conversion into adenosine, leading to reduced levels of this potent vasoconstrictor and resulting in vasodilation of the afferent arteriole, enhanced renal plasma flow (RPF), increased intraglomerular pressure and ultimately hyperfiltration[46]. Consistent with the important role of adenosine in this process, deletion of adenosine A1 receptors augments hyperfiltration and glomerular injury in animal models of diabetes[47].

Findings from studies that have examined the ability of renal hyperfiltration to predict the progression of DKD have been variable, with some demonstrating a positive association and some failing to demonstrate any association[11,52–56]. Most studies of hyperfiltration in humans have been carried out in patients with T1DM, who tend to be younger than patients with T2DM and more likely to respond normally to neurohormonal signals arising from the JGA[42]. The prevalence of renal hyperfiltration among patients with T2DM is thought to be more variable[56] as a consequence of various factors, including older age at disease onset; the presence of atherosclerotic changes that lower the responsiveness of arterioles to neurohormonal stimuli; age-related decline in GFR; longer duration of diabetes; poorer glycaemic control; and obesity.

SGLT2 inhibitors and tubuloglomerular feedback. The effect of diabetes on SGLT2 expression is unclear with some[57,58] but not all[59] studies demonstrating increased mRNA and/or protein expression of this transporter in experimental animal models of diabetes. Two human kidney biopsy studies[60,61] failed to demonstrate any increase in *SGLT2* mRNA expression but demonstrated an increase in the expression of *SGLT1* mRNA. Regardless of the effect of diabetes on sodium–glucose transporter expression, one would expect that inhibition of SGLT2 function would increase sodium delivery to the JGA and ameliorate glomerular hypertension through tubuloglomerular feedback mechanisms. Indeed, kidney-specific knockout of SGLT2 attenuated glomerular hyperfiltration and hypertension in diabetic mice but did not prevent kidney hypertrophy[62]. Furthermore, SGLT2 inhibitors reversed glomerular hyperfiltration and prevented the development of DKD in rodent models of diabetes[63,64]. Similarly, an 8-week course of empagliflozin in patients with T1DM reduced GFR in those with hyperfiltration but had no effect on GFR in those with normal filtration[42].

By increasing sodium delivery to the macula densa[50], SGLT2 inhibitors generate signals that induce afferent arteriolar vasoconstriction, decrease RPF, reduce glomerular hypertension and, on a long-term basis, attenuate progression of DKD. Consistent with these effects,

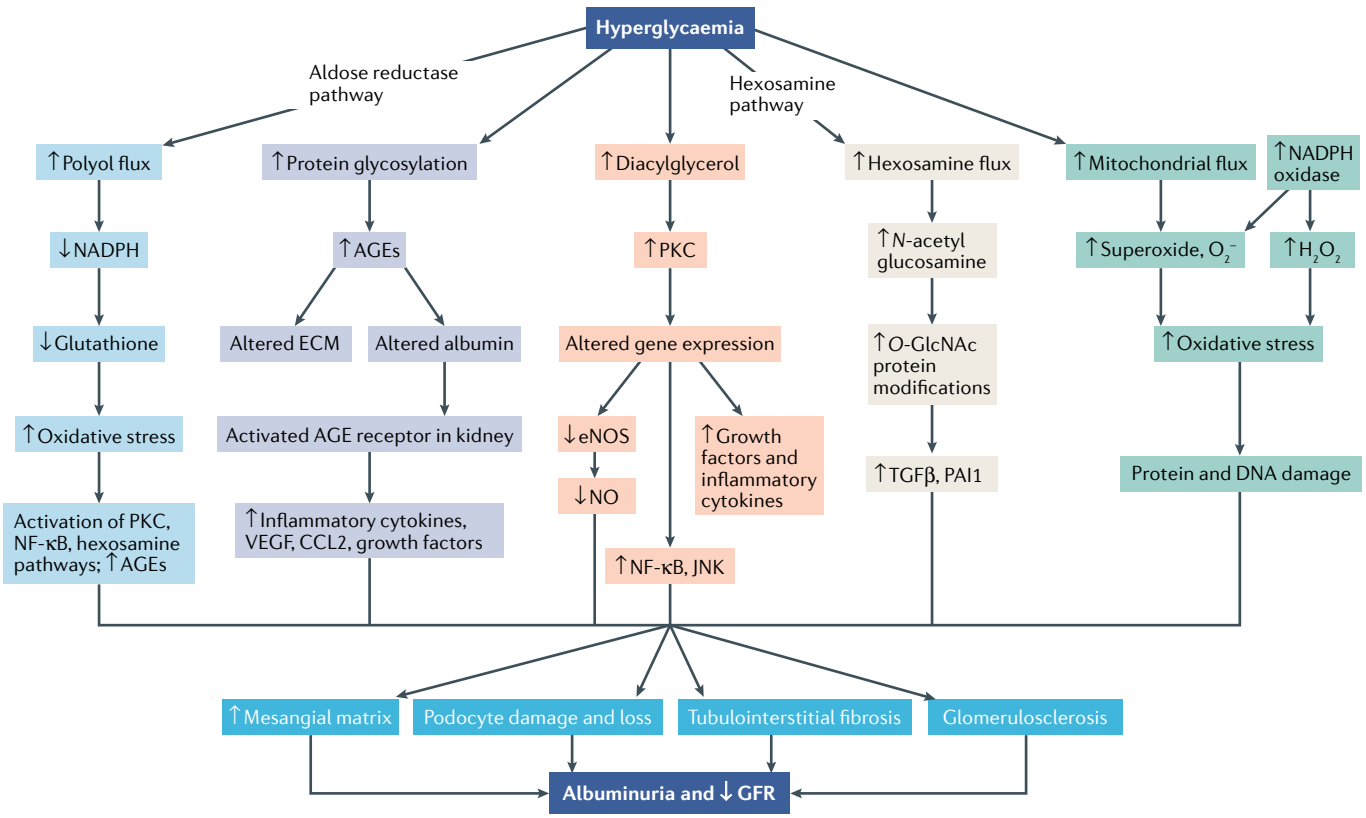

Fig. 2 | **Effect of hyperglycaemia on the kidney.** Hyperglycaemia activates multiple intracellular and biochemical pathways within the kidney that collectively contribute to the development of glomerulosclerosis, mesangial expansion, podocyte loss and tubulointerstitial damage. The clinical manifestation of these effects is a progressive decline in kidney function, accompanied by macroalbuminuria. In the presence of high intracellular glucose levels, aldose reductase reduces glucose to sorbitol via the polyol pathway, with the consumption of NADPH preventing the generation of reduced glutathione, a critical antioxidant. Hyperglycaemia also causes protein glycosylation and an increase in advanced glycosylation end products (AGEs), which activate AGE receptors in the kidney. Increased diacylglycerol activates protein kinase C (PKC) and atypical isoforms of PKC deleteriously affect gene expression, resulting in the activation of inflammatory and growth-promoting pathways. Hyperglycaemia also increases hexosamine pathway activation, resulting in the production of *N*-acetyl glucosamine and *O*-GlcNAc, which modifies serine and threonine residues of transcription factors, causing pathological changes in gene expression. Excessive flux of glucose through the mitochondrial electron transport chain generates reactive oxygen species, causing protein and DNA damage. CCL2, C-C motif chemokine 2; ECM, extracellular matrix; eNOS, endothelial nitric oxide synthase; GFR, glomerular filtration rate; NO, nitric oxide; TGFβ, transforming growth factor-β; VEGF, vascular endothelial growth factor.

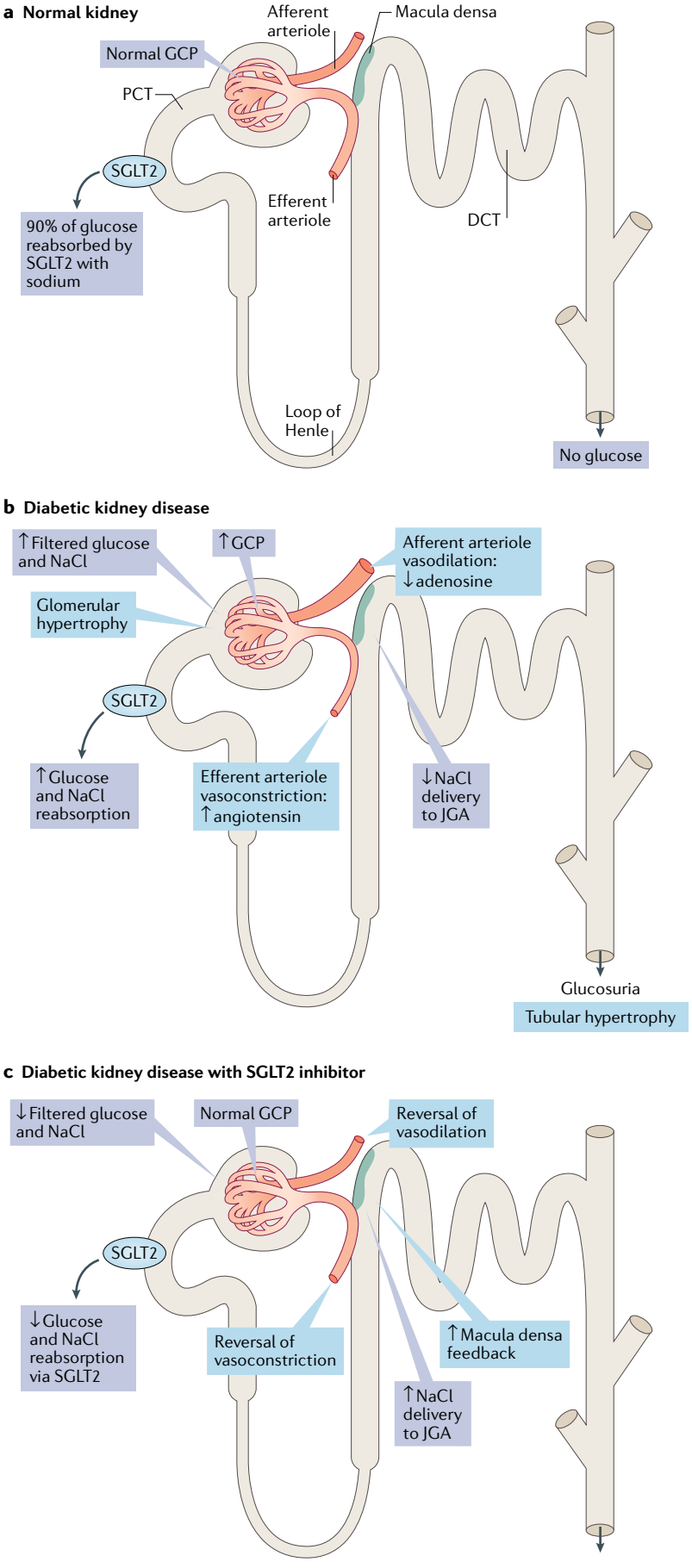

Fig. 3 | **The effect of diabetes mellitus and SGLT2 inhibitors on tubuloglomerular feedback. a** | Sodium delivery to the macula densa cells of the juxtaglomerular apparatus (JGA) has a central role in the regulation of glomerular filtration rate (GFR) and glomerular capillary pressure (GCP) through the phenomenon of tubuloglomerular feedback. Reduced NaCl delivery to the macular densa, as occurs with intravascular volume contraction, increases GFR through the local release of renin and/or angiotensin, leading to constriction of the efferent arteriole and by decreasing the release of the potent vasoconstrictor adenosine, which lead to vasodilation of the afferent arteriole. Conversely, increased NaCl to the macula densa, as occurs with intravascular volume expansion, inhibits the release of renin and/or angiotensin and stimulates the release of adenosine, thereby reducing GFR. **b** | Hyperglycaemia and diabetes increase the filtered load of glucose and NaCl, leading to enhanced glucose and sodium reabsorption by the sodium–glucose co-transporter 2 (SGLT2) transporter in the early proximal tubule. This enhanced reabsorption leads to decreased delivery of NaCl to the macula densa cells of the JGA and is interpreted by the JGA as a decrease in circulating vascular volume and renal under-perfusion. The renal response is afferent arteriolar vasodilation and efferent arteriolar vasoconstriction, resulting in increased intraglomerular pressure, GFR and glomerular pressure. On a long-term basis, the glomerular hypertension promotes glomerulosclerosis. **c** | SGLT2 inhibitors block the reabsorption of glucose and sodium in the proximal tubule, thereby enhancing delivery of NaCl to the JGA, reversing these pathophysiological changes, reducing intraglomerular pressure and preserving renal function. DCT, distal convoluted tubule; PCT, proximal convoluted tubule.

injection of the non-selective SGLT2–SLGT1 inhibitor phlorizin into the Bowman's space of diabetic rats normalized the sodium concentration at the macula densa and reversed glomerular hyperfiltration without altering the blood glucose concentration[51]. In mice with streptozotocin-induced diabetes (a model of T1DM), empagliflozin decreased afferent arteriolar diameter and hyperfiltration in association with increased urinary adenosine excretion[65]. Blockade of adenosine signalling obliterated the haemodynamic effect of empagliflozin, whereas blockade of nitric oxide (NO) and prostanoids had no effect. In young, otherwise healthy patients with T1DM and hyperfiltration, SGLT2 inhibition similarly reduced renal hyperperfusion and hyperfiltration in association with an increase in urinary adenosine excretion[42]. However, the renoprotective mechanisms of SGLT2 inhibitors reported in cardiovascular outcome studies[5–7], CREDENCE[8] and DAPA CKD[9] are likely to be different from those observed in animal models of T1DM and in studies of humans with T1DM, given that these trials involved patients with T2DM who — as mentioned earlier — are typically older and less likely to be hyperfiltering than patients with T1DM.

This proposal is supported by a 2020 study[66], which in contrast to findings from an earlier study in which SGLT2 inhibition in patients with T1DM caused vasoconstriction of the afferent arteriole, leading to a decrease in intraglomerular pressure and hyperfiltration[42], found that SGLT2 inhibition in patients with T2DM reduced

GFR and filtration fraction without increasing renal vascular resistance, suggesting a role for post-glomerular vasodilation rather than pre-glomerular vasoconstriction. The divergent findings from these studies could result from differences in the patient populations in terms of their age and obesity status; however, structural and functional differences between DKD in T1DM and T2DM also exist. The most important structural changes of T1DM DKD occur within the glomeruli, whereas tubulointerstitial lesions are more prominent in patients with T2DM[67]. As mentioned earlier, glomerular hyperfiltration is more common in patients with T1DM than in patients with T2DM[56] in whom GFR is normal or reduced owing to an age-related decline in GFR[68], although this observation does not exclude the presence of hyperfiltration at the single nephron level. The difference in renal histopathology and baseline renal haemodynamics could explain the varied responses to SGLT2 inhibitors between the two studies and suggests that the pathophysiological abnormalities responsible for DKD in T1DM and T2DM might be very different. In addition, differences in the treatment regimens in the two studies also may have contributed to their different findings. Insulin, which was given to patients in the T1DM study, is a potent vasodilator and patients who received insulin[47] were characterized by low pre-glomerular resistance and high renal perfusion. By contrast, patients in the T2DM study received metformin[66]; these patients had a lower baseline GFR and RPF and a higher renal vascular resistance, possibly restricting the ability of SGLT2 inhibitor therapy to constrict the afferent arteriole. Most of the patients in the T2DM study were also treated with RAAS blockers, whereas RAAS inhibitors were an exclusion criterion in the study of patients with T1DM. Because SGLT2 inhibitors stimulate the RAAS[42,66], the absence of RAAS blockers would leave RAAS stimulation unopposed in patients with T1DM, although the sensitivity of the efferent arteriole to angiotensin II in T2DM versus T1DM is unclear. In addition, patients in the T2DM study were more obese than patients in the T1DM study. As fatty kidney and lipotoxicity are well-established pathogenic factors for the development of kidney disease, baseline differences in kidney fat content, as well as the 2.9 kg weight loss induced by dapagliflozin in patients with T2DM, could have influenced the response to SGLT2 inhibition.

The tubule growth factor hypothesis

Tubular and glomerular hypertrophy are characteristic features of DKD and are predictors of decline in GFR and poor renal outcomes[69–73]. Poor glycaemic control is closely associated with renal hypertrophy, and intensive glycaemic control with insulin for as little as 3 months can normalize kidney size and elevate GFR in patients with T1DM[74].

The increase in proximal tubule size in DKD enhances tubule reabsorptive capacity, which, as mentioned above, reduces sodium delivery to the JGA, leading to local activation of the RAAS, efferent arteriole vasoconstriction, increased intraglomerular pressure and glomerular hyperfiltration. Angiotensin II is a potent growth factor that contributes to glomerular hypertrophy independent of its vasoactive effects[75]. According to the law of Laplace, the pressure within the glomerulus is proportional to its radius. Thus, irrespective of the aetiology of the glomerular hypertrophy, once established it will be a self-perpetuating cause of glomerular hypertension, which subsequently contributes to the development of glomerulosclerosis.

Whether the changes in sodium reabsorption and tubular hypertrophy are primary events or develop secondary to vascular events and/or in response to the actions of growth factors is unknown. As pointed out by others[50], normal tubuloglomerular feedback would be expected to induce a decline in fractional sodium reabsorption as GFR increases. The existence of glomerular hyperfiltration in the context of increased sodium reabsorption[50,51] is, therefore, consistent with the notion that the increase in sodium reabsorption in the proximal tubule is a primary event. Support for this hypothesis comes from a study[47] in which perfusion of single nephron proximal tubules with increasing concentrations of glucose increased not only glucose reabsorption but also sodium reabsorption. However, this proposal does not exclude the possibility that other factors may also contribute to tubule hypertrophy in diabetes[76,77].

In rat models of diabetes, tubule hypertrophy precedes glomerular hypertrophy and inhibition of tubule hypertrophy with an inhibitor of ornithine decarboxylase (the rate-limiting enzyme in polyamine synthesis, which has a key role in tubule hypertrophy) prevents glomerulosclerosis, glomerular hypertrophy and development of DKD[78]. In rats with streptozotocin-induced diabetes, inhibition of ornithine decarboxylase also prevented the increase in proximal tubule sodium reabsorption[78], suggesting that tubule hypertrophy, regardless of whether it is a consequence of enhanced glucose–sodium reabsorption or growth factor stimulation, has an important role in the development of glomerular hypertrophy, hyperfiltration and DKD. These observations also raise the possibility that factors released by the proximal tubule might contribute to glomerular hypertrophy.

Multiple growth factors, including insulin-like growth factor 1 (IGF1), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) have been implicated in proximal tubule hypertrophy and, thus, could contribute to the increase in proximal tubule sodium reabsorption[79]. Transforming growth factor β1 (TGFβ1) is secreted by the proximal tubules and has a pivotal role in the early hypertrophic growth response (at approximately day 4) in rats with streptozotocin-induced diabetes[79] by inducing cell proliferation, migration, differentiation and extracellular matrix (ECM) turnover[80]. TGFβ levels correlate with proteinuria and HbA_{1c}[81], and are elevated in the glomerulus during early[81] and late stages[82] of T1DM and T2DM. Use of neutralizing anti-TGFβ antibodies or antisense mRNA in diabetic mice[83] reduced mesangial matrix expansion without affecting blood pressure or blood glucose levels. However, chronic treatment of patients with a TGFβ antibody failed to alter the progression of DKD[84] — a finding that is not surprising given the multiple pathogenic factors involved in DKD development and progression.

Connective tissue growth factor (CTGF) is another tubule-derived peptide that has been implicated in the pathogenesis of DKD[85]. CTGF stimulates cell adhesion and migration, augments the production and deposition of ECM proteins[86] and promotes tissue fibrosis by activating intracellular signalling molecules in mesangial cells such as receptor tyrosine kinases and TGFβ[87]. Downregulation of CTGF ameliorates progression of DKD in mouse models of T1DM and T2DM[88] and, in a phase I study of patients with DKD associated with either T1DM or T2DM, administration of an anti-CTGF monoclonal antibody decreased microalbuminuria[89]. Future studies are likely to identify additional growth-promoting factors that contribute to glomerular hypertrophy and glomerular hypertension.

SGLT2 inhibitors and tubule growth. By directly inhibiting sodium and glucose reabsorption in the proximal tubule, SGLT2 inhibitors normalize tubuloglomerular feedback signals and mitigate hyperfiltration, as described in detail elsewhere[90]. Whether SGLT2 inhibitors affect levels of growth factors such as TGFβ and CTGF is unknown and warrants future study.

Kidney hypoxia

Kidney hypoxia is a characteristic feature of DKD and has been implicated in the development and progression of kidney disease[91,92]. Sufficient oxygen delivery to the kidney is critical to generate the ATP required for transporter function. Oxygen consumption by the kidney per gram of tissue is very high, second only to that of the heart (2.7 mmol/kg per min versus 4.3 mmol/kg per min, respectively)[93,94]. To meet this demand, renal blood flow is high — approximately 25% of cardiac output. Increased filtration and reabsorption of sodium and glucose in the diabetic kidney requires additional ATP, the vast majority of which is generated via aerobic metabolism[95]. The ability of the kidney to enhance RPF and increase oxygen delivery is limited, leading to a mismatch between oxygen demand and supply, predisposing the diabetic kidney to hypoxia (FIG. 4). Consistent with this sequence of events, animal models of diabetes demonstrate an increase in renal oxygen consumption in cortical segments by 40% and in the collecting duct segment by 16%[96].

In diabetic mice, kidney hypoxia precedes the onset of albuminuria[97] and correlates with reduced GFR[97,98]. Use of BOLD-MRI has demonstrated renal hypoxia in patients with diabetes and in patients with non-diabetic CKD, in whom it correlates inversely with GFR[99]. Endothelial dysfunction also contributes to the hypoxic environment in diabetes, triggering a fibrotic response that leads to loss of peritubular capillaries and ischaemic injury[100]. In addition, hypoxia provides a signal for the recruitment of inflammatory and immune cells[101], leading to ECM accumulation and fibrosis[102]. In animal models of DKD, the expression of hypoxia-inducible factor 1α (HIF1α) is increased and correlates with the severity of glomerulosclerosis[103–105]. Increased HIF1α normally activates the transcription of genes involved in erythropoietin synthesis, cellular energy production and defence against oxidative stress such as *HMOX1*, which encodes haem oxygenase 1 (REFS[106,107]). In DKD, however, HIF-regulated genes, including erythropoietin, are not increased for unknown reasons, resulting in decreased numbers of red blood cells and further reductions in oxygen delivery to the kidney[106,107]. The resulting exacerbation of hypoxia in turn stimulates the production of fibrotic and inflammatory molecules such as TGFβ and TNF.

SGLT2 inhibitors and hypoxia. Given the need for increased ATP and oxygen consumption to drive sodium–glucose reabsorption in the proximal tubule, we would expect that SGLT2 inhibitors would decrease oxygen requirements and improve tissue oxygenation in the kidney (FIG. 4). Indeed, a study from the 1990s found that administration of phlorizin to rats with streptozotocin-induced diabetes normalized renal oxygen consumption[51]. However, a later study found that phlorizin improved cortical oxygenation but reduced medullary oxygenation[108]. This decrease in medullary oxygenation most likely results from the increased delivery of sodium to the thick ascending limb where it is reabsorbed by the Na^+-K^+-$2Cl^-$ transporter, although the relevance of this effect to the development of DKD is unclear and warrants further investigation. Of note, the acute administration of phlorizin may not reflect the effect of chronic administration of an SGLT2 inhibitor on renal oxygen content. For example, SGLT2 inhibitors suppress levels of hepcidin, stimulating erythropoiesis independent of any effects on ferroportin[109]. These actions would be expected to reduce hypoxic stress, which, on a long-term basis, could prevent or slow the progression of DKD.

Moreover, SGLT2 inhibitors increase the production of ketones[25,26], which are a more efficient source of energy than glucose[110] and are freely taken up and oxidized by the kidney in proportion to their plasma concentration[110,111]. The oxidation of 1 mol of β-hydroxybutyrate generates 20 molecules of ATP and consumes 8 atoms of oxygen (phosphorous/oxygen (P/O) ratio = 2.50), whereas the oxidation of 1 mol of the free fatty acid (FFA) palmitate generates 105 molecules of ATP and consumes 46 atoms of oxygen (P/O ratio = 2.33)[110,112,113]. Therefore, a complete switch from fatty acid to ketone oxidation would increase renal oxygen efficiency by 9–10%, thereby reducing renal hypoxia. Studies in patients with diabetes are needed to further assess the effects of SGLT2 inhibitors on hypoxia in DKD.

Podocyte loss and albuminuria

Both charge and size limit the filtration of albumin through the glomerular capillary wall[114,115]. In patients with DKD, increased albumin excretion is mainly the result of alterations in the glomerular filtration barrier. Podocytes, which are anchored to the glomerular basement membrane, have a pivotal role in preventing the passage of albumin into the glomerular filtrate. Decreased podocyte number, podocyte detachment from the glomerular basement membrane and loss of heparan sulfate — the glycocalyx component that imparts charge selectivity to the glomerular filtration barrier — correlate directly with UAE rate in both

BOLD-MRI
Blood oxygen level-dependent (BOLD)-MRI represents a non-invasive technique for assessing renal hypoxia by measuring tissue oxygen bioavailability through measurements of relative changes in deoxyhaemoglobin.

T1DM and T2DM[116–118] (FIG. 5). Podocyte damage and apoptosis in DKD may occur in response to various stimuli. As discussed below, podocyte injury can result from the accumulation of lipid and toxic lipid metabolites in podocytes, as has been demonstrated in patients and animal models[119–121]. Further, accumulation of fatty acids in podocytes has been linked to the development of insulin resistance in vitro[122]. Insulin signalling via insulin receptors expressed by podocytes is also important for podocyte survival[123] — a finding that assumes added significance as podocytes are terminally differentiated cells[124]. In addition to glomerular injury, tubulointerstitial injury contributes to albuminuria by decreasing tubular uptake of filtered albumin and impairing albumin degradation in the tubules[125]. The presence of albumin within the glomerular filtrate is thought to elicit a sclerotic response that results in mesangial proliferation and glomerulosclerosis[126,127], as well as tubular injury[128]. Thus, albuminuria is not only a clinical manifestation of DKD but also a perpetuator of the disease[129].

SGLT2 inhibitors, podocyte loss and albuminuria. We have shown that dapagliflozin enhances insulin sensitivity in patients with T2DM[25]. If SGLT2 inhibitors are also able to improve insulin sensitivity in podocytes, they could potentially prevent podocyte injury and reduce albuminuria in DKD. In diabetic and non-diabetic mice with protein overload-associated kidney injury induced by bovine serum albumin[130], administration of dapagliflozin limited glomerulosclerosis, prevented podocyte dysfunction and loss, and reduced proteinuria. Of particular importance, this study[130] identified SGLT2 in podocytes and showed that mRNA and protein expression of podocyte SGLT2 increased following bovine serum albumin injection. This novel observation provides a potential mechanism by which SGLT2

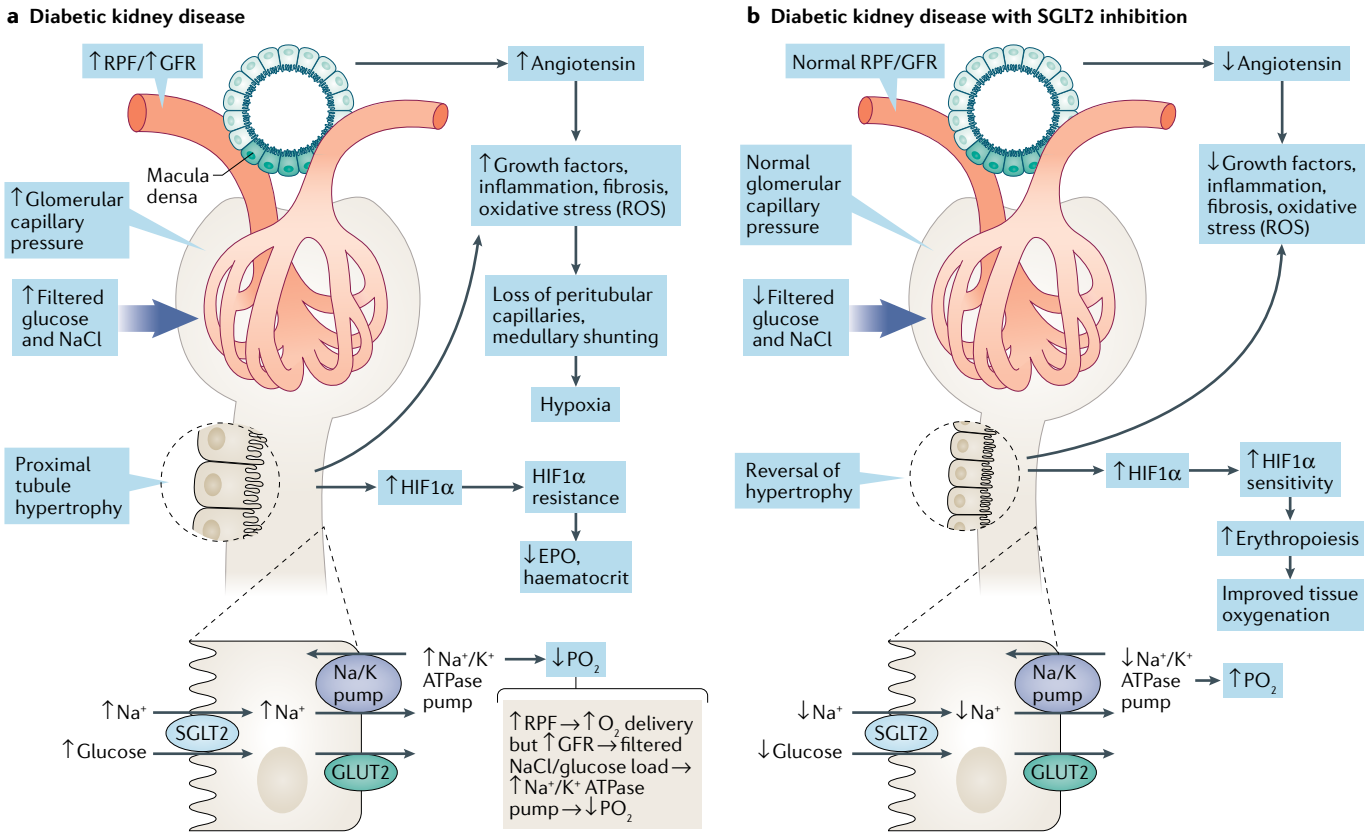

Fig. 4 | **The effect of diabetes and SGLT2 inhibitors on kidney oxygen delivery and demand. a** | The diabetic kidney is characterized by tissue hypoxia owning to an imbalance between oxygen consumption and oxygen delivery. The increase in plasma glucose concentration and elevated renal plasma flow (RPF) and glomerular filtration rate (GFR) results in an increase in the filtered load of glucose and sodium, leading to augmented glucose and sodium reabsorption by sodium–glucose co-transporter 2 (SGLT2) in the proximal tubule. The increase in intracellular sodium must be transported out of the proximal tubule by the Na^+/K^+ ATPase pump, an energy demanding process that requires oxygen and predisposes to hypoxia, which promotes fibrosis, the release of growth factors and inflammatory molecules, and the generation of reactive oxygen species (ROS). In addition, loss of peritubular capillaries can lead to shunting of blood flow by the medullary region of the kidney, further contributing to renal hypoxia. The normal renal response to tissue hypoxia is to increase hypoxia-inducible factor 1α (HIF1α), which activates the transcription of genes involved in erythropoietin (EPO) synthesis, cellular energy production and defence against oxidative stress. In diabetic kidney disease, HIF1α increases, but HIF-regulated genes, including *EPO*, do not increase, leading to a reduction in red blood cell count and reduced oxygen delivery to the kidney. **b** | SGLT2 inhibitors block glucose and sodium absorption by SGLT2 in the proximal tubule, decreasing activity of the Na^+/K^+ ATPase pump and, thus, the increased oxygen demand. The glucosuric effect reduces the plasma glucose concentration, while normalization of tubuloglomerular feedback reduces GFR, both contributing to a decrease in the filtered load of glucose and sodium and further reducing the need for oxygen to drive the Na^+K^+ ATPase pump. Correction of renal hypoxia increases sensitivity to HIF1α, leading to an increase in EPO levels and erythropoiesis. Amelioration of hypoxia attenuates inflammation, fibrosis and the generation of ROS.

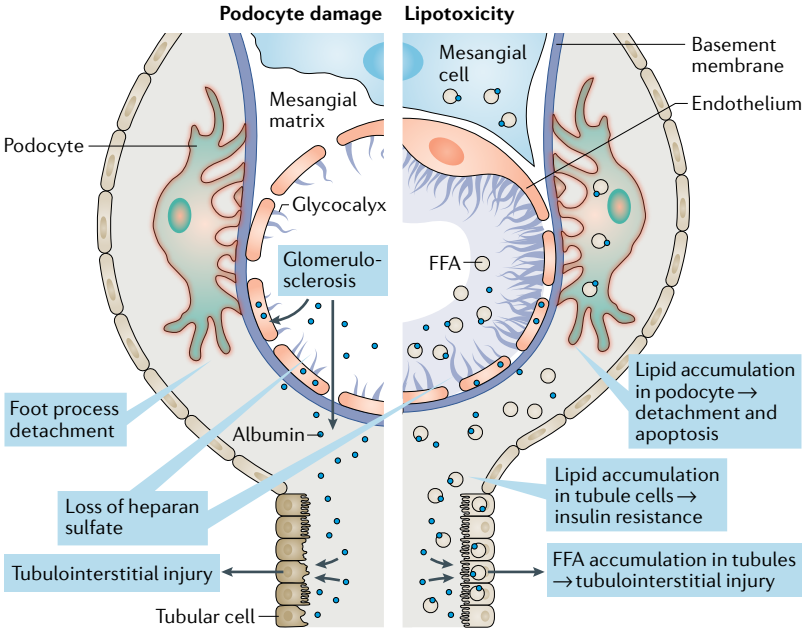

Fig. 5 | **Podocyte injury in diabetic kidney disease.** Podocyte integrity is essential for maintenance of the normal glomerular filtration barrier. Podocyte injury is a characteristic feature of diabetic kidney disease, leading to albuminuria, which is toxic to the kidney, causing glomerular sclerosis and tubulointerstitial injury. In the setting of obesity, podocytes, as well as mesangial, endothelial and tubule epithelial cells, become overloaded with free fatty acids (FFAs) and other lipid moieties, leading to lipotoxic injury. Thus, both lipotoxicity and albumin-induced glomerular and tubular damage contribute to the progressive demise of kidney function.

inhibitors might directly target the kidney to prevent DKD. Empagliflozin has also been shown to augment podocyte autophagy, preventing podocyte effacement, detachment and death in a mouse model of DKD[131]. The increase in podocyte autophagy was associated with reduced mesangial expansion, improved glomerular histology and decreased albuminuria. Finally, by causing a shift from whole-body glucose utilization to fatty acid oxidation[25], SGLT2 inhibitors may decrease podocyte lipid content, thereby promoting podocyte health and reducing albuminuria.

Obesity and lipotoxicity

Obesity, independent of diabetes, dyslipidaemia and hypertension, is associated with the development of CKD[119,132] characterized by glomerular hypertrophy, mesangial expansion, glomerulosclerosis and podocyte injury[133]. As obesity is a key risk factor for T2DM, it is not surprising that obesity can contribute to the decline in GFR in individuals with diabetes. Consistent with this notion, in a renal biopsy study of 620 individuals with typical clinical and laboratory features of DKD, only 37% had histological features of DKD, 36% had a diagnosis of non-diabetic kidney disease and 27% had histological features of DKD plus non-diabetic renal disease[134]. It is possible that the atypical histological findings were, in part, related to the concomitant presence of obesity.

Lipotoxicity is a well-established cause of insulin resistance in muscle and liver[135] and of β-cell failure[136]. Renal lipotoxicity, as evidenced by the accumulation of lipid in podocytes, mesangial cells and proximal tubule epithelial cells, is a contributing aetiological factor to diabetes-related, as well as obesity-related, nephropathy[119,132,135] (FIG. 5). Podocyte injury, characterized by lipid vacuolization, decreased podocyte density and foot process effacement, is a characteristic finding in both diabetes-related and obesity-related nephropathy[119,120,132,133,137]. Typical glomerular findings of obesity-related kidney disease, including podocyte injury, are observed in mice fed a high-fat diet long before the onset of overt diabetes[138,139]. Podocytes are especially sensitive to lipotoxic injury and endoplasmic reticulum stress. Normal podocyte function depends on the integrity of lipid rafts, the composition of which is altered by diabetes[140]. Lipid accumulation in podocytes causes insulin resistance and cell death[141]. Of note, deletion of the insulin receptor in podocytes leads to the development of renal lesions that mimic DKD[124]. Individuals with obesity have elevated plasma levels of FFAs regardless of diabetes status. However, FFAs in the circulation are bound to albumin and restricted from glomerular passage. Podocyte injury allows albumin-bound FFAs to be filtered and access to the proximal tubule. In the glomerulus, FFAs can induce glomerular injury and, following reabsorption by the proximal tubule, they can promote tubulointerstitial disease[142,143]. Mitochondrial oxidation of fatty acids stimulates the production of reactive oxygen species (ROS), further contributing to tubular damage and apoptosis[144]. Furthermore, increased transport of albumin-bound fatty acids into the proximal tubules induces endoplasmic reticulum stress[145]. Inhibition of VEGFB, which is known to control lipid accumulation in muscle through the regulation of fatty acid transport, reduces renal lipotoxicity, resensitizes podocytes to insulin signalling and prevents the development of diabetic glomerulopathy in mice with DKD[146].

SGLT2 inhibitors, obesity and lipotoxicity. SGLT2 inhibitors uniformly produce a weight loss of 2–3 kg during the initial 6–12 months of therapy[25]; however, it is unlikely that this modest reduction in weight contributes substantially to the reversal of lipotoxicity and prevention of DKD observed in clinical studies. We and others[25,26,147] have shown that SGLT2 inhibitors cause a shift in substrate utilization from glucose to FFAs. This shift would induce a reduction in intracellular levels of toxic lipid metabolites, such as fatty acyl CoAs, diacylglycerol and ceramides. Decreased levels of these lipids in podocytes, mesangial cells and proximal tubular cells would be expected to reduce oxidative stress, endoplasmic reticulum stress and pro-inflammatory and fibrotic processes.

Inflammation

Inflammatory mechanisms also contribute to the development and progression of DKD through increased monocyte and macrophage adherence to endothelial cells and overexpression of pro-inflammatory cytokines and chemokines[148–152]. Deletion of C-C motif chemokine 2 (CCL2; also known as MCP1) and intracellular adhesion molecule 1 attenuate macrophage infiltration and reduce kidney injury in mouse models of T1DM and T2DM[153].

In humans with DKD, accumulation of glomerular macrophages correlates strongly with the progression of kidney impairment[154]. Infiltrating macrophages release lysosomal enzymes, NO, ROS, TGFβ, VEGF and cytokines such as TNF, IL-1 and IFNγ[149], which can accelerate the development and progression of DKD. Using bone marrow chimeric mice, it has been shown that macrophages contribute directly to DKD, potentially by altering the viability of podocytes[155]. Genetic deficiency or pharmacological blockade[156] of C-C chemokine receptor type 2 (CCR2) blocks macrophage recruitment to the kidneys in mouse models of diabetes, attenuating albuminuria and blood urea nitrogen levels and improving histological parameters. In patients with T2DM and proteinuria, treatment with the CCR2 inhibitor, CCX140-B, for 52 weeks significantly reduced UAE[157].

Activation of the IκB–NF-κB signalling pathway in muscle contributes to peripheral tissue insulin resistance in patients with T2DM[158]. NF-κB is a potent regulator of inflammation and stimulates the transcription of genes that encode cytokines, adhesion molecules and angiotensinogen — all of which have been implicated in DKD[36,159]. Bardoxolone — another inhibitor of NF-κB — has demonstrated renoprotective effects in patients with DKD, but a major clinical trial of bardoxolone in patients with DKD was stopped early because of adverse events[160].

The JAK–STAT signalling pathway is a major transducer of inflammatory signals and is activated by many cytokines, chemokines and growth factors[161]. In experimental models of DKD, this pathway is activated in podocytes[162], mesangial cells[163] and tubule cells[164]. In kidney biopsy samples from patients with DKD, JAK–STAT expression is increased[162,164] and correlates with the severity of kidney disease[165], whereas overexpression of JAK–STAT in mice worsens DKD[166]. In a study of 129 patients with diabetes and mean GFR of 45 ml/min/1.73 m^2, administration of the JAK1 and JAK2 inhibitor baricitinib for 24 weeks reduced the urinary albumin-to-creatinine ratio by 41%[167]. Suppressors of cytokine signalling (SOCS1 and SOCS3) normally act as part of a negative-feedback system to inhibit activation of JAK–STAT signalling. Reduced expression of SOCS1 and SOCS3 in experimental models of DKD[168] could therefore contribute to activation of the JAK–STAT pathway in mesangial cells[166,169].

SGLT2 inhibitors and inflammation. Although inflammatory markers have not been reported in cardiovascular and renal outcome trials of SGLT2 inhibitors[5–8], modest reductions in circulating inflammatory markers, such as IL-6, TNF and IFNγ, have been reported in small SGLT2 inhibitor trials[170,171]. SGLT2 inhibitors have also been shown to decrease markers of inflammation and oxidative stress in animal models of DKD, associated with improvements in histological parameters[172–174]. In macrophages isolated from patients with T2DM, empagliflozin attenuated ATP-induced secretion of IL-1β and activation of the NLRP3 inflammasome, secondary to an upregulation of the ketone body β-hydroxybutyrate[173]. Transcriptomic analysis of plasma biomarkers in patients with T2DM showed a decrease in levels of genes encoding TNF receptor 1, matrix metalloproteinase 7 (MMP7) and fibronectin 1 in patients treated with canagliflozin for 2 years compared with levels in patients treated with glimepiride[174]. These findings suggest that SGLT2 inhibitors may attenuate molecular processes related to inflammation, ECM turnover and fibrosis.

In line with this proposal, a study in rats with cardiac infarction found that dapagliflozin markedly suppressed collagen synthesis by activating M2 macrophages[175]. Moreover, a study of cultured human cardiac fibroblasts found that empagliflozin reduced collagen matrix formation by inhibiting the expression of profibrotic markers and TGFβ1 (REF.[176]). The relevance of these findings in cardiac tissue to the kidney are unknown but are worthy of exploration.

Endothelial dysfunction and oxidative stress

Endothelial dysfunction, characterized by reduced bioavailability of NO and increased oxidative stress, is a hallmark feature of T2DM and DKD[177]. NO is produced from arginine by NO synthases (NOSs) and arginases. In response to chronic hyperglycaemia, endothelial NOS is impaired, resulting in the production of ROS and oxidative stress in lieu of NO[178,179]. Low levels of endothelial NOS have been shown to exacerbate DKD[180,181]. Activity and expression of arginase 2 are increased in kidneys of mice with DKD[182] and pharmacological blockade or genetic deficiency of arginase 2 confers kidney protection[183] via an eNOS-dependent mechanism[184].

Impaired NOS activation results in the accumulation of superoxide and other ROS, which induce oxidative stress, leading to damage of essential cellular components, protein and DNA[185]. In the kidney, these processes can lead to glomerulosclerosis and tubular fibrosis[186]. The deleterious effects of superoxide and ROS are mediated via activation of protein kinase C, the NF-κB pathway, hexosamine metabolites and advanced glycosylation end products[187]. Although antioxidants have been used in animal models of DKD with some benefit[36], the results have been unimpressive, most likely owing to the existence of multiple redundant mechanisms underlying DKD and because the more proximal inciting event — hyperglycaemia — is not adequately corrected.

SGLT2 inhibitors and endothelial dysfunction. A metabolomics analysis of kidney biopsy samples from patients with DKD demonstrated upregulation of genes involved in the NOS pathway in response to dapagliflozin treatment[188], indicating that SGLT2 inhibitors might improve endothelial function. However, findings from clinical studies of patients treated with SGLT2 inhibitors are conflicting. In the randomized, controlled DEFENCE study of 80 patients with early-onset T2DM, dapagliflozin improved endothelial function (as measured by change in flow-mediated dilation)[189]. By contrast, in the randomized, controlled EMBLEM trial of 117 patients with T2DM and established cardiovascular disease, empagliflozin showed no beneficial effect on endothelial function[190]. Findings from preclinical studies are also unclear. In vitro exposure of cultured human proximal tubule cells exposed to tofogliflozin suppressed the induction of ROS by glucose[191]. In mouse models

of diabetes, SGLT2 inhibitors also protected against endothelial dysfunction, but their vasodilatory effect was found to be secondary to reduced inflammation and amelioration of oxidative stress[192]. Support for the notion that endothelial protective effects of SGLT2 inhibitors may be indirect comes from an in vitro study, where impairment of NO-mediated contraction and relaxation of cardiac microvascular endothelial cells by TNF was reversed by the addition of empagliflozin[193].

Mitochondrial injury

Mitochondrial dysfunction also has an important role in the pathogenesis of DKD[194,195]. Proximal tubules and podocytes are particularly vulnerable to mitochondrial dysfunction leading to oxidative stress, energy depletion, impaired energy-dependent repair mechanisms and cell death[195,196]. PGC1α, a master regulator of mitochondrial biogenesis, is abundantly expressed in the kidney[197] and activates transcription factors such as NRF1, NRF2, ERRα and PPARα, which, in turn, regulate the transcription of genes required for mitochondrial biogenesis[150]. Metabolic analyses of urine samples and kidney biopsy samples have identified a signature of mitochondrial dysfunction associated with DKD[198]. Overexpression of PGC1α promotes the recovery of mitochondrial and cellular function following renal injury[199,200], but this effect has not specifically been examined in models of DKD.

ROS are also generated by NADPH oxidases (NOXs) and nitric oxidase synthetases[201], both of which are decreased in diabetic kidneys[198,201,202]. Inhibition of NOX1/NOX4 with GKT137831 ameliorates DKD in murine models[203,204], but a clinical trial in human DKD failed to demonstrate a reduction in albuminuria[205].

SGLT2 inhibitors and mitochondrial injury. The effect of SGLT2 inhibition on mitochondrial function has so far received little attention. However, a 2020 study identified metabolic pathways in patients with T2DM that were modulated by dapagliflozin, including transcripts indicative of improved mitochondrial function[188]. Moreover, findings from in vitro and in vivo studies suggest that SGLT2 inhibitors may improve mitochondrial function in renal tubules[206,207].

Fibrosis

Structural changes in glomeruli are primarily responsible for the progressive decline in GFR that occurs in patients with DKD. However, structural changes are also evident within the tubules and interstitium, including thickened tubule basement membranes, tubule atrophy, interstitial fibrosis and chronic inflammation[208]. Fibrosis is characterized by the deposition of collagen, laminin, fibrillary proteins, activated myofibroblasts and inflammatory cells[209] and these changes are evident in rodents following short-term exposure to hyperglycaemia[210,211].

A variety of factors contribute to the development of fibrosis in the damaged kidney. TGFβ is an important mediator of fibrosis and its expression has been shown to be consistently elevated in the kidneys of humans and animal models of DKD[212]. CTGF is a downstream effector of TGFβ and promotes fibrosis; increased levels of CTGF are associated with the development of kidney dysfunction in patients with T2DM[213]. MMPs are elevated in individuals with DKD, are associated with interstitial fibrosis and correlate inversely with kidney function[214]. Lipids[132] and the RAAS also promote kidney fibrosis[215]. RAAS inhibitors can reduce interstitial fibrosis in rat models of diabetes and attenuate levels of profibrotic factors such as TGFβ11, PDGF, CTGF, MMP2 and TIMP1 (REF.[216]). In cultured human proximal tubule cells, hyperglycaemia directly stimulated the production of growth factors (PDGF and CTGF) and increased the fibrotic transformation of renal fibroblasts — effects that were blocked by RAAS inhibitors[216].

SGLT2 inhibitors and fibrosis. Support for antifibrotic effects of SGLT2 inhibitors in DKD comes from both in vivo[64] and in vitro[217] studies. In obese, diabetic *db*/*db* mice, dapagliflozin caused dose-dependent reductions in albuminuria, mesangial expansion and interstitial fibrosis[64]. Histologically, macrophage infiltration and the expression of TGFβ1 and inflammatory genes were also reduced. Similarly, treatment of human cultured proximal tubule cells with empagliflozin decreased production of the inflammatory molecules NF-κB and TLR4 induced by high glucose[217]. The reduction in inflammatory factors in both of these studies indicates that the renal antifibrotic effects of SGLT2 inhibitors are likely secondary to their anti-inflammatory actions. In a rat model of myocardial infarction, dapagliflozin reduced the number of myofibroblasts and inflammatory molecules and the extent of myocardial fibrosis[175]. In line with these findings, empagliflozin reduced the expression of TGFβ, collagen deposition and cardiac fibrosis in a mouse model of diabetes[218]. Empagliflozin also inhibited TGFβ1-induced fibroblast activation and suppressed profibrotic markers such as type 1 collagen, CTGF and MMP2 in cultured human cardiac fibroblasts[219]. These findings provide evidence for a direct antifibrotic effect of SGLT2 inhibitors on cardiac myocytes and are consistent with the observed antifibrotic effects of SGLT2 inhibitors in the kidney.

Sodium–hydrogen exchanger

Activation of sodium–hydrogen exchangers (NHEs) in the heart (NHE1) and kidney (NHE3) have been implicated in the development of heart failure and DKD, respectively[220,221]. NHE1 is ubiquitously expressed and is the major NHE isoform in the heart, whereas NHE3 is primarily expressed in kidney and gastrointestinal cells. In heart failure, NHE1 activity is increased in cardiomyocytes, leading to increased cytosolic sodium and calcium concentrations.

In the kidney, NHE3 and NHE1 are expressed in the proximal tubule[222,223] and in mesangial cells[224] and are upregulated in the diabetic state in vivo and by hyperglycaemia in vitro. Much of the sodium reabsorption that occurs in the proximal tubule occurs in exchange for H^+ and is mediated by NHE3 — the activity of which is increased by luminal glucose[223]. SGLT2 is colocalized with NHE3 in the kidney[225] and their activity is linked via the accessory protein membrane-associated protein 17 (MAP17)[226], such that increased activity of one

transporter increases the activity of the other and vice versa. Thus, increased activity of NHE3 could contribute to the development of glomerular hyperfiltration and mesangial proliferation. Of interest, NHE3 abundance and transport activity are also increased in the proximal tubule of rats with heart failure[227].

SGLT2 inhibitors and the sodium–hydrogen exchanger. In rabbits, rats and mice, SGLT2 inhibitors inhibit the NHE1 exchanger to decrease cytoplasmic sodium and calcium, increase mitochondrial calcium and improve myocardial contractility[228]. Of note, cardiomyocytes do not express SGLT2 (REF.[229]), and, therefore, the inhibitory effect of SGLT2 inhibitors on NHE1 most likely represents an off-target effect.

In the kidney, inhibition of SGLT2 decreases NHE3 activity, whereas knockout of NHE3 decreases SGLT2 expression and inhibits the induction of natriuresis by SGLT2 inhibition[230,231]. Phlorizin, an inhibitor of SGLT2 and SGLT1, reduces NHE3 activity in perfused proximal tubules of non-diabetic rats, leading to reduced reabsorption of bicarbonate even in the absence of luminal glucose[230]. The subsequent increase in sodium delivery to the macula densa would be expected to reset the tubuloglomerular balance, reduce intraglomerular pressure and decrease hyperfiltration. Inhibition of NHE3 in mesangial cells[224] represents another potential mechanism by which SGLT2 inhibitors could ameliorate DKD by preventing glomerulosclerosis. Although the interaction between NHE3 and SGLT2 provides a potential mechanism via which SGLT2 inhibitors may provide renal protection in the context of diabetes, further study is needed to establish this link.

Autophagy

Autophagy is a cellular recycling process by which damaged or aged organelles and proteins are degraded by proteolytic enzymes within lysosomes and recycled as an energy source and is essential for normal cell function and survival[232]. In states of energy deprivation, such as starvation, increased autophagy is essential for energy salvage. Autophagy is also important for the maintenance of mitochondrial homeostasis. The presence of excess energy in the context of diabetes shifts the balance of mitochondrial fission and mitochondrial fusion towards fission with an increased number of dysfunctional mitochondria in muscle[233] and promotes oxidative stress and inflammation. DKD is characterized by mitochondrial dysfunction and structural changes consistent with sustained fission and impaired autophagy[234,235].

Impaired autophagy of various cell types has been documented in kidney diseases, including DKD[236,237]. Mice with podocyte-specific knockout of key autophagy molecules develop proteinuria, glomerulosclerosis and kidney failure[236], whereas exposure of podocytes[238] and proximal tubule cells[239,240] to high glucose concentrations impairs autophagy and promotes apoptosis. Somewhat counterintuitively, enhanced autophagy also promotes apoptosis in mesangial cells in diabetic rodents[237]. Of note, mTORC1 is increased in diabetic mice and inhibition of mTORC1 stimulates autophagy and prevents podocyte loss, glomerulosclerosis and proteinuria[237,241]. Adenosine monophosphate-activated protein kinase (AMPK) also promotes autophagy and could represent a target to prevent DKD[237].

SGLT2 inhibitors and autophagy. In obese, diabetic *db*/*db* mice, empagliflozin activates autophagy in podocytes, prevents mesangial expansion and reduces UAE[131]. Empagliflozin also prevented the histological changes of DKD in mice with streptozotocin-induced diabetes[242]. In human renal proximal tubule cells cultured under high glucose conditions, empagliflozin improved mitochondrial biogenesis, normalized the expression of proteins involved in mitochondrial fusion and fission, and enhanced autophagy in association with mTOR inhibition[242]. Empagliflozin also attenuated the mitochondrial production of ROS and apoptotic markers and normalized AMPK activity. Of interest, canagliflozin, but not empagliflozin or dapagliflozin, has been shown to activate AMPK in cultured hepatocytes by inhibiting complex I of the mitochondrial respiratory chain[243].

Through their glucosuric effects, SGLT2 inhibitors induce an acute reduction in plasma glucose, and with it insulin concentrations, leading to decreased glucose entry into muscle cells. To meet their energy requirements, myocytes switch to fat as an alternative source of energy[244,245]. Of note, dapagliflozin reduces the rate of ATP synthesis from substrates of complex I and complex II in muscle, causing a state of energy deprivation[244]; this reduction in muscle ATP synthesis correlates strongly with an increase in plasma ketone concentration[244]. The deficit in energy metabolism induced by dapagliflozin has the potential to slow the progression of DKD by normalizing autophagy[234,235]. Although not yet demonstrated, it is reasonable to hypothesize that energy deprivation, as occurs with SGLT2 inhibitor therapy, would improve autophagy and restore the balance between mitochondrial fission and fusion.

Conclusions

The pathogenesis of DKD, like diabetes itself, is multifactorial and has important clinical implications. The most definitive means of preventing DKD is to maintain HbA_{1c} <6.5% (upper limit of the prediabetic range) and ideally within the normal range (<5.7%). However, once histological and clinical evidence of DKD becomes manifest, targeting multiple pathophysiological disturbances simultaneously is likely to be needed to slow or prevent progression to kidney failure. SGLT2 inhibitors affect a number of pathophysiological processes that underlie the development and progression of DKD. The multifactorial actions of this new class of drugs probably underlies their renoprotective effects as demonstrated by a growing number of clinical trials. The use of SGLT2 inhibitors, in combination with RAAS inhibitors and blood pressure-lowering and glucose-lowering medications, represents a promising approach to preventing the development and progression of DKD. We have no doubt that further insights into the mechanisms underlying DKD will stimulate the development of novel therapeutic approaches in the future.

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Mitochondrial fission
The splitting of mitochondria into two, which, together with mitochondrial fusion, is required for mitochondrial homeostasis.

Mitochondrial fusion
The combining of two mitochondria, which, together with mitochondrial fission, is required for mitochondrial homeostasis.

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