Sodium glucose cotransporter 2 and the diabetic kidney

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**Purpose of review**
Reabsorption of glucose in the proximal tubule occurs predominantly via the sodium glucose cotransporter 2 (SGLT2). There has been intense interest in this transporter as a number of SGLT2 inhibitors have entered clinical development. SGLT2 inhibitors act to lower plasma glucose by promoting glycosuria and this review aims to outline the effect on the diabetic kidney of this hypoglycaemic agent.

**Recent findings**
This review provides an overview of recent findings in this area: the transcriptional control of SGLT2 expression in human proximal tubular cells implicates a number of cytokines in the alteration of SGLT2 expression; experimental data show that SGLT2 inhibition may correct early detrimental effects of diabetes by reducing proximal tubular sodium and glucose transport, suggesting a possible renoprotective effect independent of the glucose lowering effects of these agents; and the nonglycaemic effects of SGLT2 inhibitors may have an impact on renal outcomes.

**Summary**
The available clinical evidence shows consistent reduction in glycaemic parameters and some evidence suggests additional effects including weight loss and mild blood pressure reduction. There are some side effects that warrant further investigation and establishing whether SGLT2 inhibition provides a renal benefit relies on future long-term studies with specific renal end-points.

**Keywords**
diabetic nephropathy, proximal tubule, sodium glucose cotransporter 2

**INTRODUCTION**
Diabetic nephropathy is the most common cause of end-stage kidney disease in the world. The prevalence of some degree of renal involvement in diabetic patients reaches 40% [1], with significant progression to end-stage disease. A treatment gap exists in the current prevention and treatment of diabetic nephropathy, and implementing current best practice reduces this gap at best by 30%. Subsequently, there is significant interest in the role of sodium glucose cotransporter 2 (SGLT2) inhibitors, with many drugs from this class in phase III clinical trials and some awaiting approval for licensing. These drugs act by blocking glucose reabsorption in the proximal tubule, resulting in increased glycosuria and a subsequent reduction in blood sugar levels. Whereas the use of SGLT2 inhibitors has been confirmed in clinical trials [2,3] to result in improvement in glycaemic control, the renal effects are less well studied. They include potential benefits both from improved glycaemic control and from effects independent of glycaemic control such as weight reduction, lowering of blood pressure (BP) with improved cardiovascular outcomes and unique renoprotective benefits. There are, however, also potential risks related to increased glycosuria including higher frequency of urinary infections, genital fungal infections, volume depletion and off target effects. This review will outline the current evidence in regard to both the renal risks and benefits of SGLT2 inhibitors.

**GLUCOSE AND SODIUM ABSORPTION BY THE KIDNEY**
Nearly 180 g of glucose is filtered in the glomerular filtrate everyday. The kidney absorbs most of this glucose with less than 0.5 g excreted in the urine.
Hormones, autacoids, neurotransmitters and growth factors

KEY POINTS

- SGLT2 inhibitors have shown consistent glycaemic benefits.
- They have anticipated adverse effects and the link to malignancy needs further exploration.
- Experimental evidence suggests that they can be renoprotective.
- Only long-term clinical trials will prove the real renal benefits and explore links to unanticipated effects.

The sodium dependent glucose transporters (SGLT), located on the apical side of the proximal tubule cell, are able to accumulate glucose within the cell against a concentration gradient by transporting glucose concurrently with sodium. A sodium concentration gradient is provided by a Na–K-adenosine triphosphatase pump located on the basolateral side that pumps sodium out of the cell. Glucose is then passively transported across the basolateral side of the cell via facilitative glucose transporters (GLUT) into the interstitium. In the early segments of the proximal tubule, SGLT2 on the apical membrane is coupled with GLUT2 on the basolateral side, and it reabsorbs up to 90% of filtered glucose under normoglycaemic conditions [5]. By blocking the reabsorption of glucose and sodium in the proximal tubule, SGLT2 inhibitors act to reduce blood glucose levels by enhancing glucose excretion, and promote a natriuretic and diuretic action which should normalize the altered sodium handling seen in diabetes.

To demonstrate the glucose reabsorption capacities of SGLT2 further, SGLT1 gene knockout mice show nearly 97% reabsorption of filtered glucose compared with wild-type mice [6]. By contrast, it has been shown in SGLT2 gene knockout animals that reabsorption of the filtered glucose is incomplete, ranging from 10 to 60% depending on the amount of filtered glucose. Although this demonstrates that SGLT1 transporters are not able to completely make up for a lack of SGLT2, there is some compensatory uptake by SGLT1 in this situation [7**, which has implications for patients treated with SGLT2 inhibitors. In addition, a third type of sodium glucose transporter, SGLT3, has been identified and evaluated over the last few years. It has been recently confirmed to be a glucose-stimulated sodium transporter, although it does not transport glucose [8*]. It could potentially contribute to the altered sodium handling seen in diabetes. However, it is unclear at present how SGLT2 inhibitors might affect the expression and activity of SGLT3 in the human kidney, an area for future study.

There has been increasing work done in recent times in the area of SGLT2 expression and function, which may potentially impact on the importance and efficacy of SGLT2 inhibitors in diabetic patients. Diabetes is associated with an increased ability to reabsorb filtered glucose, due to an increase in the tubular transport maximum for glucose [9]. This is clearly counterproductive for the diabetic patient, both serving to increase plasma glucose levels and exposing the proximal tubular cells to an increased concentration of glucose. This increase in glucose reabsorption may in part relate to an alteration in SGLT2 expression seen in diabetic conditions and possibly also to a change in SGLT1 expression. Proximal tubular cells obtained from the urine of diabetic patients have shown increased expression of SGLT2 [5] and studies in obese Zucker rats have shown that diabetes causes increased RNA expression of SGLT2 and SGLT1 in the kidney [10]. A variety of factors have been linked to the alteration in expression of SGLT1 and 2, including HNF1α and SGK1 [11], but more recently our group has confirmed upregulation of expression of SGLT2 when proximal tubular cells are exposed to transforming growth factor β (TGFβ), a profibrotic cytokine [12]. Interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) have been shown to increase SGLT2 expression in cultured kidney cell lines after exposure for 96–120 h [13*] and a pathway for high glucose-induced increased SGLT2 expression has been demonstrated via protein kinase A (PKA) and protein kinase C (PKC) dependent pathways [14,15**]. There is also evidence of interaction between the sodium glucose cotransporters and the renin–angiotensin–aldosterone system. It has been shown in animal studies that losartan, an angiotensin receptor blocker (ARB), reduces SGLT2 expression in diabetic rats on normal and high salt diets [16]. This intriguing connection suggests another possible mechanism for the beneficial role of ARBs in diabetic nephropathy although the effect of ARBs on tubular reabsorption has not been tested.

Finally, evidence also exists in regard to the effect of glucose on the expression and location of the facilitative glucose transporters, which usually reside in the basolateral membrane of the proximal tubular cell. It has been shown in diabetic rats that GLUT2 expression increases in diabetes and translocates to the luminal surface of the proximal tubular cell, playing a role in increased glucose reabsorption [17].

Clearly, although SGLT2 is the predominant glucose transporter in the proximal tubule, its
expression and that of the other glucose transporters may be altered in the diabetic milieu, having implications for the efficacy of selective SGLT2 inhibitors, perhaps evidenced by their lack of complete glucose blockade despite increasing doses. To that end, there is renewed interest in dual SGLT1/SGLT2 blockade with one drug, LX4211, currently in clinical trials [18].

**PROXIMAL TUBULAR GLUCOSE TRANSPORT AND THE CONTRIBUTION TO DIABETIC NEPHROPATHY**

Diabetic nephropathy results from high glucose mediated inflammation and altered sodium handling that eventually results in fibrosis. There are both glomerular and tubulointerstitial damage, although the decline in renal function parallels more closely the degree of tubulointerstitial damage [19]. High glucose is directly responsible for the changes seen in diabetic nephropathy, and improved glycemic control has been demonstrated to slow the progression of the disease [20]. However, there are two processes that contribute to the disorder of diabetic nephropathy that may be expected to be altered by SGLT2 inhibitors, independent of their effect on glucose lowering (Fig. 1).

The proximal tubular cell secretes inflammatory molecules and growth factors in response to high glucose. This results in activation of an inflammatory cascade and recruitment of macrophages with propagation of hypertrophy and interstitial fibrosis [21*]. The most important mediator of this pathogenesis is TGFβ, which promotes fibrosis and epithelial to mesenchymal transdifferentiation [22]. There are a host of other inflammatory mediators and growth factors involved in this complex process [23]. It is possible that a reduction in glucose transit through the proximal tubular cells may reduce proximal tubular cell-induced inflammation and fibrosis in diabetic nephropathy. Indeed, our group has shown that SGLT2 inhibition in immortalized proximal tubular cell lines (HK2 cells) reduced high glucose induced Toll-like receptor 2 and 4 as well as nuclear factor kappa B (NFκB) and activator protein 1 (AP1) expression, which are important for renal injury (Fig. 1).
inflammatory and fibrotic mediators in diabetic nephropathy [12].

Another important aspect of diabetic nephropathy is hyperfiltration-associated renal injury. Glomerular hyperfiltration associated with enhanced sodium and glucose reabsorption in the proximal tubule occurs quite early in the disease process [24] and plays an important role in diabetic nephropathy [25]. Increased proximal tubular sodium reabsorption results in decreased distal delivery of sodium to the macula densa, which regulates tubuloglomerular feedback [26]. By inhibiting sodium reabsorption in the proximal tubule and by thereby increasing sodium delivery to the juxtaglomerular apparatus, it might be expected that the glomerular hyperfiltration would be reversed. Indeed, phlorizin, a nonselective SGLT inhibitor, has been shown to abrogate the development of hyperfiltration in whole animal studies, and in single nephron studies animals treated with phlorizin have a reduction in sodium reabsorption and normalization of glomerular filtration rate (GFR), disproportionate to the improvement in plasma glucose [24]. This finding has been recently confirmed using a rat model and a selective SGLT2 inhibitor, and extended to show that the effect is sustained with chronic SGLT2 blockade [27], although there may be a relative increase in sodium reabsorption in Henle’s loop resulting in a slightly reduced response in single nephron GFR (SNGFR) compared with acute SGLT2 blockade.

GENETIC DEFECTS IN SODIUM GLUCOSE COTRANSPORTER 2 EXPRESSION

Familial renal glycosuria (FRG) occurs due to several genetic mutations ranging from missense and nonsense mutations to small deletions and splicing mutations that are known to result in loss of SGLT2 function. Their inheritance has variously been described as autosomal recessive [28] or codominant inheritance with variable penetrance [29]. To date, 49 different SLC5A2 mutations have been reported in association with FRG. However, a recent study detailing FRG in 23 unrelated children showed at least one consistent mutation in all individuals, suggesting that genetic heterogeneity may not be prevalent [30*]. The degree of glycosuria depends on the pattern of gene inheritance, with heterozygotes having far less glycosuria than individuals with homozygous inheritance, and ranges from a few grams to more than 120 g per day [31,32]. This condition seems to be well tolerated by the individuals affected, apart from activation of the renin-angiotensin system due to volume depletion in some individuals and occasional aminoaciduria.

The literature is consistent with FRG being a largely benign condition with no serious adverse consequences.

However, the recent development of the Sweet pee mouse model may be cause for some concern. This model, which carries a nonsense mutation in the Slc5a2 gene, has a loss of function of the SGLT2 protein that mimics patients with similar SLC5A2 mutations. As expected, when diabetes mellitus is induced, these mice demonstrate increases in glycated haemoglobin that are less than their wild-type counterparts, but a little unexpectedly, Sweet pee mice suffer from growth retardation, and increased infections and mortality [33*]. While this might appear to be a cautionary message in regard to the use of SGLT2 inhibitors, it should be remembered that diabetes was induced with streptozotocin, which mimics a type 1 diabetic phenotype, not the type 2 diabetes for which SGLT2 inhibitors are intended.

Finally, a recent study has explored the impact of common SGLT2 variants on glucose homeostasis in nondiabetic individuals, finding alterations in glucose and insulin concentrations dependent on SGLT2 activity [34*]. This concept of genetic variability causing alterations in SGLT2 function within patients receiving SGLT2 inhibitors opens the door for further pharmacogenomic studies to clarify the role of SGLT2 inhibitors in treating diabetic patients.

SODIUM GLUCOSE COTRANSPORTER 2 INHIBITORS AND RENAL ENDPOINTS

There are at least 12 SGLT2 inhibitors in various stages of clinical development. Some of these are in phase III trials and two drugs are awaiting approval from licensing authorities. The most advanced among the SGLT2 inhibitors are dapagliflozin and canagliflozin. The other prominent drugs being developed under this category include empagliflozin, sergliflozin, ipragliflozin, tofagliflozin and luseogliflozin. These drugs are attractive as antidiabetic agents because of their insulin independent action and a reduced incidence of hypoglycaemia.

The SGLT2 inhibitors have been shown to reduce fasting plasma glucose and haemoglobin A1c (HbA1c) levels in treatment naive diabetic patients as monotherapy [3] and also as add on therapy to patients already on insulin [2,35] and metformin [36,37]. The degree of drop in HbA1c varies from 0.58 to 1% in these trials. Tighter control of diabetes has been shown to reduce the incidence of diabetic nephropathy on long-term follow-up of the intensive glucose control cohort in the UK Prospective Diabetes Study [20]. Intensive blood
SGLT2 and the diabetic kidney

Komala et al.

Glucose control has also been shown to reduce the incidence of diabetic nephropathy in a cohort of patients with type 2 diabetes over a 5-year period in the Action in diabetes and vascular disease: Preterax and Diamicron controlled evaluation trial [38]. Hence, SGLT2 inhibitors should have a direct renal benefit due to their glycaemic effects [20]. Ideally, hard end points of renoprotection should be demonstrated by long-term preservation of GFR and prevention of albuminuria, but to date that data is not available. A nonselective SGLT inhibitor, T-1095, has been shown to reduce HbA1c and reduce the degree of microalbuminuria in yellow KK mice, an obese type II model with insulin resistance [39]. Streptozotocin-induced diabetic rats treated with T-1095 showed no increase in urinary albumin levels and kidney weight, when compared with diabetic control rats [40]. This drug has also been shown to preserve glomerular structure and reduce mesangial expansion in diabetic animals [41].

In clinical trials, dapagliflozin has shown transient decline in the GFR on initiation of treatment, which normalizes in patients who have normal baseline renal function [42**]. However, in a 52-week placebo-controlled trial in patients with moderate renal dysfunction (GFR >30 and <60), this initial drop remains stable without correction during the duration of treatment, although there was improvement in albuminuria [43]. In a 26 week, phase III placebo-controlled trial in patients with moderate renal dysfunction, canagliflozin at varying doses showed mild worsening of creatinine levels (9 and 10% vs. 4%), although the albumin creatinine ratio (ACR) and HbA1c showed improvement [44]. Finally, dapagliflozin has been reported to be less effective in lowering HbA1c in patients with a reduction in renal function, as might be expected given that the effect of the drug requires ample GFR [43].

Further long-term studies with hard renal endpoints are required to establish the renal benefits of SGLT2 inhibition.

**Nonglycaemic Benefits**

Whereas improved glycaemic control is likely to translate into improvement in renal outcomes, the SGLT2 inhibitors also have a number of nonglycaemic effects that may contribute to renal benefit, including effects on weight, BP, lipids and uric acid.

Patients receiving SGLT2 inhibition undergo weight loss [2,45], which initially may represent loss of fluid due to the drugs’ diuretic effect but in the long-term is caused by a loss of subcutaneous fat secondary to urinary loss of calories [36]. Body weight loss appears to plateau after several months of treatment, perhaps due to a compensatory increase in calorie intake [3,36,46].

As outlined above, SGLT2 inhibition is likely to cause natriuresis and diuresis that would likely be associated with a reduction in BP. SGLT2 inhibition has been shown to prevent BP rises in diabetic rats maintained on a high-salt diet [47] and there has been a reduction in seated systolic BP of 4.4 mmHg and diastolic BP of 2.1 mmHg among pooled data from dapagliflozin’s clinical trials, without any significant increase in postural hypotension [35,36].

Canagliflozin has been noted to raise both HDL and LDL in one of the trials [37] and dapagliflozin has been noted to raise HDL [48]. The dual SGLT1/ SGLT2 blocker, LX4211 has been shown to reduce triglyceride levels in a 28-day study in diabetic patients and may be related to an increase in glucose like peptide-1 release [18]. Although these results are interesting, they may reflect the associated weight loss these agents induce and further studies are required.

Finally, dapagliflozin has been shown to reduce serum uric acid levels with no significant change in electrolytes [49]. This may occur due to an increase in uric acid excretion via one of two mechanisms: increased flux of sodium through sodium-dependent phosphate transporters that simultaneously serve as urate transporters into the urine; or increased reabsorption of glucose via GLUT9 that exchanges glucose for uric acid [50]. Regardless, with elevated levels of uric acid being linked to progression of chronic kidney disease [51], reduction of levels may serve to improve renal outcomes in diabetic patients.

**Renal Risks**

Most of the clinical trials show increased incidence of genital fungal infections [36], although urinary infections were only minimally different [35,36] and were largely managed with conventional medications and did not require cessation of SGLT2 inhibitors. Interestingly, in studies wherein dapagliflozin was added to metformin, there was no difference in the incidence of urinary tract infections between the two arms [36], whereas in those patients for whom the drug was supplementary to insulin there was an increase in infection rate [35]. This difference may reflect differences in stage of the disease wherein insulin is likely to be prescribed and at which patients are more vulnerable to infection.

Given that osmotic diuresis accompanies the glycosuria and natriuresis, there have always been concerns that treatment with SGLT2 inhibition may
result in symptomatic volume depletion. Indeed, an increase in urine volume is noted both with acute and chronic SGLT2 inhibition that at most represents an increase of 400 ml per day [42**]. Although this tends to translate into mild haematoctrit increases, clinical signs of volume depletion are unusual and have generally only been noted among patients on dapagliflozin who were also being treated with loop diuretics [42**].

The major concerns raised by the US Food and Drug Administration (FDA) regarding dapagliflozin, however, were the higher incidence of bladder and breast cancer among patients receiving this medication and one probable case of drug-induced hepatitis [42**]. The FDA has recommended further trials to evaluate these specific concerns before approval. However, dapagliflozin has received a favourable decision from the Committee for Medicinal Products for Human Use of the European Medicines Agency and is awaiting approval. A pharmacovigilance plan for this drug will be implemented as part of the marketing authorization. Canagliflozin has an application submitted for approval to the FDA and also to the European Medicines Agency.

CONCLUSION

Information on role of SGLT2 in glucose and sodium transport has increased in the last 2 years. There is increasing knowledge of this transporter and the mechanisms of pharmacological inhibition of both SGLT2 and SGLT1 [52]. A large amount of data from clinical trials is available in the public domain and the drugs will soon enter the pharmacological market. These trials have confirmed glycaemic benefits and minor adverse effects which were expected based on animal studies and early clinical trials. However, they have also brought to focus other concerns regarding malignancy and drug reactions. These concerns will only be allayed by longer clinical trials and postmarketing monitoring.

Most of the trials have focused on the classic outcome measures of antidiabetic drugs, namely improved glycaemia. Renal disease is one of the major microvascular complications of diabetes, and to date, whereas short-term studies have been reassuring in regard to renal safety with these drugs, there has been a lack of long-term data clarifying renal benefit. Given the glycaemic benefit of these agents and the other nonglycaemic effects, such as weight loss, BP control and reduced uric acid levels, in combination with the long-term effects of reduced glucose reabsorption through the proximal tubular cell and alteration in sodium handling, it would be anticipated that long-term trials will yield a benefit.

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Conflicts of interest

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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 134).

15. Ghezzi C, Wright EM. Regulation of the human Na+-dependent glucose cotransporter hSGLT1. Am J Physiol Cell Physiol 2012; 303:C348–C354. This article demonstrates that SGLT2 activity is increased through PKC and PKA pathways and postulates different mechanisms by which this is accomplished.


34. Enigk U, Breitfeld J, Schlenitz D, et al. Role of genetic variation in the human sodium-glucose cotransporter 2 gene (SLC5A2) in glucose homeostasis. Pharmacogenomics 2011; 12:1119–1126. This article describes the role of common SGLT2 variants in glucose and insulin levels in nondiabetic patients and suggests the importance of potential difference in response to SGLT2 inhibitors in management of diabetes.


