Beyond Glycosuria: Exploring the intrarenal effects of SGLT\(_2\) inhibition in diabetes

M. C. Thomas\(a,b,*\), K. Jandeleit-Dahm\(b\), F. Bonnet\(c\)

\(^a\)Baker IDI Heart & Diabetes Institute, 75 Commercial Rd, Melbourne, Australia, 3004
\(^b\)Monash University, Dept. of Epidemiology and Preventive Medicine, Melbourne, Australia
\(^c\)Inserm UMR 991, service endocrinologie-diabétologie, CHU Rennes, université Rennes 1, 2 rue du Thabor, CS 46510, 35065 Rennes cedex, FRANCE

Abstract

For millennia, the syndrome that has become known as diabetes was considered to be primarily a disease of the urinary system and, by association, of dysfunction in the kidneys (recognized as the source of urine). In the last decade, there has been renewed interest in the role of the kidneys in the development and maintenance of high glucose levels. This has led to the development of novel agents to inhibit sodium-glucose cotransporter 2 (SGLT-2) as a means to control glucose levels and augment calorie-wasting leading to weight loss. However, beyond actions on glycaemic control, inhibition of proximal glucose absorption via SGLT-2 has significant direct effects to attenuate hyperfiltration and reduce renal hypertrophy. Increased distal sodium delivery may also act to suppress the intrarenal renin-angiotensin-aldosterone system, although systemic activity may be modestly increased due to osmotic diuresis. Reducing proximal glucose reabsorption may also protect the tubular cells from exposure to excess glucose and glucose-induced reactive oxygen species. On the other hand, distal glucose delivery following inhibition of SGLT-2 may increase glycogen deposition, the significance of which is unclear. However, subjects with familial glycosuria appear to have a benign renal prognosis. Some studies have demonstrated significant reductions in albumin excretion in various experimental models and as post-hoc observations in clinical trials. Whether these reflect renoprotection or are simply the result of intraglomerular haemodynamic changes remains unclear. Although promising, such actions remain to be established by comprehensive clinical trials with a renal focus, many of which are currently in progress.

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1. Background

The link between diabetes and the pancreas is only 120 years old. For millennia before then, the syndrome that has become known as diabetes was considered to be primarily a disease of the urinary system and, by association, of dysfunction in the kidneys (recognized as the source of urine). Ancient Egyptian writings first record a syndrome of excessive urination and wasting. In traditional Ayurvedic medicine, diabetes was known as ‘madhumeha’—from ‘madhu’ (meaning sweet) and ‘meha’ (meaning excessive urination) – and a diagnosis was suspected by noting how black with attracted flies was the urine of certain individuals. Across many parts of the world there were professional ‘water tasters’ (the historical equivalent of pathology laboratories) employed to diagnose diabetes. The term ‘mellitus’ (Latin for ‘honey’) was added by these tasters, who noted that sticky diabetic urine was “wonderfully sweet, as if it were imbued with honey or sugar”. Indeed, even in the pancreas/insulin-centric diabetic practices of the modern era of medicine, the elimination of glycosuria has been considered a therapeutic priority in the management of diabetes, as both a means to prevent or reduce symptoms as well as an indicator of systemic control of the disease. However, over the past decade, there has been renewed interest in the role of the kidneys in the development and maintenance of high glucose levels, and the potential for interventions targeting these pathways to improve glycaemic control. In addition, beyond glycaemic control, there are emerging data that these pathways may also have an impact on the development and progression of diabetic kidney disease [1,2]. The present review assesses the data pertaining to the direct effects of sodium-glucose cotransporter-2 (SGLT\(_2\)) inhibition of the kidneys, as well as the potential clinical relevance of such actions. A detailed discussion of the actions of SGLT\(_2\) inhibitors on glycaemic control, blood pressure and weight loss, all of which may indirectly benefit the kidneys, may be found in other reports in this issue.

2. Glucose reabsorption pathways in the healthy kidney

Glucose is freely filtered at the glomerulus, meaning that approximately 180 g of glucose enters the primary urine and flows into the proximal tubule. Almost all of this filtered load (>99.9%) is then reabsorbed by sodium-coupled transport across
the brush-border membrane of the proximal tubule and then returned to the circulation by glucose transporter 2 (GLUT2) and, to a lesser extent, GLUT1 [1,2]. The driving force behind glucose cotransport is an electrochemical gradient for sodium, which is established and maintained by basolateral Na+/K+ ATPase [1,2]. More than 90% of the glucose initially filtered is reabsorbed in a 1:1 ratio with sodium by a low-affinity, high-capacity system controlled by SGLT2 in the early convoluted segment of the proximal tubule (s1) [1]. Intracellular glucose then induces a conformational change in GLUT2, allowing it to passively move across the cell membrane into plasma, where it returns to the circulation. Reabsorption of the remaining luminal glucose load (in a 1:2 ratio with sodium) is performed by sodium-glucose cotransporter 1 (SGLT1), a low-affinity, high-capacity transporter further down in the straight segment of the descending proximal tubule (s3) [1,2]. So efficient is this glucose reabsorption pathway that, of the ~180 g filtered, <0.1 g finds its way into the urine of non-diabetic individuals [1-4].

Nevertheless, the capacity for renal glucose reabsorption appears to be limited. If the filtered load exceeds the capacity for glucose reabsorption, then glucose (and the water held with it) spills over into urine. This happens first in some nephrons before others, producing the characteristic splay in the relationship between glucose load and glycosuria (Fig. 1) [5]. However, a maximum reabsorption capacity is eventually reached in all nephrons, and any further increase in glucose load is then matched by an equivalent loss of glucose in urine, resulting in a linear relationship between blood glucose levels and glycosuria. The threshold at which glucose spills into urine is, on average, around 10-11 mmol/L [6], but this can vary significantly from person to person from as little as 6 mmol/L to >14 mmol/L [7]. The key factors that determine this variability remain unclear. The renal threshold also varies within a given person at different times and with different circumstances in life [8]; for example, children have a lower glucose reabsorption capacity compared with adults [9]. French studies in the 1980s demonstrated that obesity raised the threshold for glucose reabsorption independently of its effects on glycaemic control [10]. Also, during a normal pregnancy, the tubular glucose reabsorption maximum is lowered by approximately half (to levels equivalent to those seen following inhibition of SGLT2 in clinical practice and in type A familial renal glucosuria; see below).

Some studies dispute the concept of an absolute threshold/tubular transport maximum for glucose (TmG) reabsorption, as using sensitive methods for the measurement of urinary glucose, even at euglycaemic blood glucose levels, glycosuria can still be demonstrated [11]. This is sometimes known as ‘basal’ or ‘physiological’ glycosuria, which appears to function independently of blood glucose concentration, urinary flow rate, and the renal threshold for glucose and maximum rate of tubular glucose absorption [12]. However, the potential mechanism remains to be established. Basal glycosuria may be attributable to distal tubular ‘leakage’ or antiporter activity exchanging glucose for other metabolites. Nevertheless, an alternative progressive tubular reabsorption model might better describe the observed variations in glycaemia and glycosuria following a glucose load than the threshold model of glucose reabsorption in the kidneys [13].

3. Benign familial glycosuria

The importance of tubular SGLT2 to glucose homeostasis is best illustrated by the phenotype associated with mutations of the SLCA2 gene that reduce the expression and/or activity of SGLT2 [14]. This phenotype, known as ‘benign familial glucosuria’ or ‘familial renal glycosuria’, is associated with a reduced capacity for glucose reabsorption. In type O glucosuria, the reabsorption of glucose is severely reduced or absent, meaning that otherwise normoglycaemic individuals can waste over 100 g of glucose every day. This condition is specifically discussed in detail elsewhere in this special edition. However, it is important to note that the presence of glucose in urine beyond the straight segment of the descending proximal tubule results in the accumulation of intracellular glycogen granules in the distal tubules, including the thick ascending limbs in the cortex and outer stripe of the outer medulla (the so-called Armanni-Ebstein lesion) [15]. Despite this, familial glycosuria is a generally benign condition, although dehydration and ketosis may sometimes develop during starvation or pregnancy. Notably, persistent glycosuria in this setting has no long-term adverse effects on distal tubular or renal function. These data provide support for a benign renal safety profile following pharmacological inhibition of SGLT2.

4. Glucose reabsorption in diabetes

In most (but not all) patients with diabetes, the capacity for glucose reabsorption in the proximal tubule is increased perhaps as a way to conserve energy. This may partly be due to the tubular hypertrophy and increased Na/K/ATPase activity in the diabetic kidney. In addition, the expression and activity of apical SGLT2, and basolateral glucose transporter proteins are also increased in diabetes [16]. Proximal tubular cells isolated from the urine of diabetic individuals show increased uptake of labelled glucose analogues compared with non-diabetic

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Fig. 1. Inhibition of SGLT2 shifts the relationship between plasma glucose and glucose excretion to the left, thereby reducing the threshold of glycosuria by 2 mmol/L on average.
individuals [17], demonstrating that these reabsorption pathways are more active in the setting of hyperglycaemia, paradoxically at a time when glucose levels are already elevated. An increase in glucose threshold by 2 mmol/L on average, as observed in diabetic individuals, will retain, on average, an extra 60 g of glucose every day, the caloric equivalent of a Snicker’s candy bar, and require that extra insulin be made (or given) to keep plasma glucose levels under control. In comparison, increased hepatic glucose production in diabetes is said to contribute approximately 30 g/day to the glucose load. In addition, instead of losing the calories attributable to glucose to urine, (glucose) energy is retained chiefly as body fat.

Consequently, these changes in renal glucose reabsorption are considered to significantly contribute to the maintenance of hyperglycaemia in patients with diabetes [5], and provide a strong rationale for inhibition of SGLT2, as a means to better control glucose levels while, at the same time, augmenting calorie-wasting and lowering blood pressure. Changes in glucose reabsorption may also influence glycaemic control in gestational diabetes. As noted above, during normal pregnancy, the renal glucose reabsorptive capacity decreases, possibly as a means to prevent hyperglycaemia in the setting of the naturally increased insulin resistance associated with pregnancy. Yet, it has recently been shown that women with gestational diabetes retain a higher renal glucose threshold during pregnancy, suggesting that the failure to suppress renal glucose absorption may be contributing to gestational diabetes [18].

5. Pharmacological inhibition of SGLT2

Pharmacological inhibition of SGLT2 reduces the capacity for tubular glucose reabsorption by approximately 30-50% (Fig. 1). Why a greater level of inhibition is not achieved in vivo, despite being a potent and comprehensive inhibitor in vitro, is unclear. It may be that the pharmacokinetics at the proximal tubule, including shuttling of transporters, means that, at any one time, most SGLT2 are not occupied [19]. In addition, the up-regulation of SGLT1 expression and activity also acts to reduce the excretion of filtered glucose following inhibition of SGLT2 [20]. However, this level of inhibition is sufficient to lower the threshold of spillover, thus resulting in a urinary glucose wastage in patients with diabetes of approximately 50-80 g/day. Also, these losses do not significantly wane over time, but may persist for at least 4 years. In contrast, the natriuresis induced following treatment with an SGLT2 inhibitor is transient, like that observed with thiazide natriuretics, as compensatory sodium reabsorption pathways are reset to prevent further renal sodium losses and to sustain a new balance.

6. SGLT2 inhibition and hyperfiltration

Conventional paradigms for the development of diabetic nephropathy describe an increase in estimated glomerular filtration rate (eGFR) that precedes increasing albuminuria by several years [21]. It is thought that hyperglycaemia leads to increases in glomerular capillary pressure and single-nephron GFR (SNGFR) that cumulatively increase the GFR. This is known as ‘hyperfiltration’, although it would be better thought of as recruitment of renal reserve function as, in much the same way, dietary protein loading is also known to cause hyperfiltration. In both cases, it is thought that increased proximal sodium reabsorption leads to reduced distal sodium delivery and subsequent inactivation of tubuloglomerular feedback pathways at the macula densa, leading to a proportionally greater decrease in afferent vs efferent arteriolar resistance (through the actions of adenosine, angiotensin, prostaglandins and other vasoactive mediators) and subsequent increases in glomerular capillary pressure, which mediates hyperfiltration (Fig. 2). In the case of diabetes, the mediators of increased proximal sodium absorption are many and various, and include tubular hypertrophy and increased Na/K/ATPase activity. In addition, increased glucose reabsorption, even at subthreshold glucose levels, results in augmented sodium reabsorption as a result of its coupling with glucose at SGLT2 [22]. The likely key importance of SGLT2 in this phenomenon is demonstrated by the observation that genetic deletion or pharmacological inhibition of SGLT2 is able to attenuate the hyperfiltration associated with experimental diabetes [23-25], most probably by reducing proximal sodium reabsorption and increasing distal sodium delivery, thereby triggering the macula densa to reduce SNGFR [26]. Similar reductions in hyperfiltration have also been observed following SGLT2 inhibition in a small 8-week study of patients with type 1 diabetes (T1D) [27]. In patients with type 2 diabetes (T2D), SGLT2 inhibition is associated with a modest reversible reduction in eGFR of 3-8 mL/min/1.73 m2 [28-30]. In contrast, GFR is not affected by genetic deletion or inhibition of SGLT2 in non-diabetic mice, possibly because there is little or no glycosuria as plasma glucose levels are physiological, and the enhanced sodium uptake via SGLT1 (which reabsorbs two sodium molecules

![Diagram of Tubular Mechanisms of Hyperfiltration](https://via.placeholder.com/150)

**Fig. 2. Tubular mechanisms of hyperfiltration in response to hyperglycaemia, and their amelioration following inhibition of SGLT2.**
that tubular hypertrophy is a key contributory factor to increased glucose. In fact, it may be quite the opposite; it may be an epiphenomenon associated with the induction of pathogenic changes, including thickening of the glomerular basement membrane [34,35]. This paradigm has also been used to explain the role of nephron endowment in determining the predisposition for diabetic nephropathy as well as the renoprotective effects arising from blockade of the renin-angiotensin-aldosterone system (RAAS), which reduces glomerular pressure via its selective actions on the effenter glomerular arteriole.

However, it remains controversial as to whether prevention of hyperfiltration on its own is sufficient to confer renoprotective effects in the diabetic kidney. Our studies with the Finnish Diabetic Nephropathy (FinnDiane) Study group suggest that creatinine- or cystatin-based estimates of GFR are not a predictor of the development of microalbuminuria in patients with T1D [36]. Similar results from the large-scale International Diabetic Nephropathy Study found that glomerular basement membrane width, mesangial fractional volume and mesangial matrix accumulation were not correlated with baseline renal function [37]. Consistent with these findings, diabetes-associated hyperfiltration in the same SGLT2 knockout models was prevented, although markers of renal injury, inflammation or fibrosis were not reduced [23]. Similarly, inhibition of SGLT2 with empagliflozin was able to reduce hyperfiltration, but attenuated renal injury only in proportion to glucose-lowering [38].

7. SGLT2 inhibition and tubular hypertrophy

Hyperplasia and hypertrophy of the cortical tubuli and concomitant renal enlargement is one of the earliest structural changes in the diabetic kidney, and can be observed after as little as 48 h of exposure to hyperglycaemia [39]. Whether hyperfiltration is a mediator of long-term renal damage through, for example, augmenting fibrogenesis, or accelerating senescence or mesenchymal transition of tubular cells, or is simply an epiphenomenon associated with the induction of pathogenic mediators, remains to be established. At least in experimental models of diabetes, inhibition of tubular hypertrophy appears to have renoprotective effects [40].

Proximal tubular cells do not use absorbed glucose as a metabolic substrate for energy production, and any glucose reabsorbed across the luminal membrane by SGLT1 leaves the cell across the basolateral membrane by GLUT2. This means that hypertrophy is not the direct result of taking up more glucose. In fact, it may be quite the opposite; it may be that tubular hypertrophy is a key contributory factor to increased glucose uptake in the diabetic kidney. Indeed, it has been suggested that tubular hypertrophy is very likely a compensatory response to the increased glucose load associated with hyperglycaemia (sensed by SGLT2) to reduce the risk of calorie wasting. Consistent with this hypothesis, pharmacological inhibition of SGLT2 is able to attenuate (but not abolish) renal hypertrophy associated with experimental diabetes [38,41].

8. SGLT2 inhibition and intrarenal RAAS

One of the most important pathways implicated in the development and progression of diabetic nephropathy is the RAAS. Blockade of this pathway by ACE inhibitors or angiotensin II receptor type 1 (AT1) blockers has been shown to reduce both albuminuria and the risk of end-stage renal disease (ESRD) in diabetes. Activation of RAAS in diabetes may also contribute to up-regulation of sodium-glucose transport pathways in the proximal tubule. This may be an indirect effect through the induction of growth factors and subsequent tubular hypertrophy. However, direct effects of angiotensin II through the AT1 receptor on expression of SGLT2 may also be involved. Indeed, there are data to suggest that blockade of the RAAS is able to prevent diabetes-associated up-regulation of SGLT2 expression [42].

In states of volume depletion, RAAS activation promotes salt and water reabsorption. In states of sodium and volume excess, suppression of the RAAS results in natriuresis and water loss. The chief sensor is the macula densa which, by sensing sodium concentration, is able to activate or suppress the intrarenal RAAS with regard to volume status. SGLT2 inhibition leads to modest intravascular volume depletion associated with osmotic diuresis, potentially contributing to activation of systemic RAAS in patients receiving these agents [27,43,44]. At the same time, increased delivery of sodium to the macula densa may be sensed as the opposite by the kidneys, thus triggering pathways to enhance natriuresis, including reduction of the intrarenal RAAS. How these changes may affect the progression of diabetic nephropathy is unclear. However, systemic blockade of the RAAS in most diabetic patients may turn out to be highly advantageous when initiating SGLT2 inhibition. Indeed, in one experimental study, the combination of RAAS blockade with SGLT2 inhibition was associated with additional renoprotective effects compared with either drug alone [45]. Further interventional studies are warranted to confirm these preliminary findings in diabetic patients.

9. SGLT2 inhibition and intrarenal oxidative stress

Oxidative stress is an important player in the pathogenesis of diabetic kidney disease. Diabetes is associated with the activation of enzymes that directly liberate reactive oxygen species (ROS), including nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [46,47] and xanthine oxidase. Mitochondrial dysfunction also leads to the generation of ROS in the diabetic kidney, as well as the development and progression of nephropathy. ROS have also been implicated in the induction of glucose transport pathways...
in the diabetic kidney [48]. In addition, treatment of diabetic mice with SGLT2 inhibitors reduces renal markers of oxidative stress both in vitro and in vivo, suggesting that the effects of SGLT2 inhibition extend beyond improvements in glucose control, weight loss and blood pressure reductions. However, any direct mechanism remains to be established [49,50]. Certainly, elevated uric acid levels have been identified as potential contributors to microvascular injury, partly through the induction of oxidative stress, and inhibition of SGLT2 results in modest uricosuria [51]. This is probably due to the exchange of luminal glucose for intracellular urate mediated by SLC2A9b, which becomes more active in the presence of glycosuria [52].

10. Future directions

Despite the considerable promise of renoprotective actions arising from SGLT2 inhibition in the setting of diabetes and the observed renal benefits of many different SGLT2 inhibitors in experimental diabetes, any clinically relevant actions have yet to be established [38]. A number of small short-term clinical trials have documented modest reductions in urinary albumin excretion [28,30,53,54]. However, on the whole these actions have been in proportion to reductions in hyperglycaemia and, as such, may simply be reflections of glomerular haemodynamics and unrelated to any underlying pathophysiological changes in the kidneys. However, safely protecting the kidneys against glucotoxicity could have advantages for renoprotection although, at this stage, we don’t know. A number of long-term studies in patients with chronic kidney disease (CKD) are currently underway, including the CANVAS-R (NCT01989754), the Evaluation of the Effects of Canagliflozin on Renal and Cardiovascular Outcomes in Participants with Diabetic Nephropathy (CREDENCE) (NCT02065791) and the EMPA-REG OUTCOME™ (NCT01131676) trials. Nevertheless, a key limitation of such studies is the fact that most SGLT2 inhibitors are contraindicated in patients with stage 3 CKD (moderate-to-severe renal impairment) due to the lack of glucose-lowering efficacy in this setting, as a decreased GFR means that glucose losses following SGLT2 inhibition are thereby attenuated. This underscores the fact that the diabetic patients most at risk of adverse renal outcomes and, in particular, ESRD will either not be included in these studies or may have to discontinue therapy should progressive renal impairment arise. Consequently, surrogates such as albuminuria will have to be used for patients with early renal disease, and this may or may not be adequate to reflect the true renoprotective potential of such interventions. Finally, there remains an urgent need to establish robust surrogates for progressive, but early, renal injury and the use of novel biomarkers of ESRD risk such as soluble tumour necrosis factor (TNF) -α receptor 1 [55] to assess the potential renoprotective effects of SGLT2 inhibitors.

Disclosure: The authors have received honoraria from companies involved in the development and marketing of SGLT2 inhibitors, including Bristol-Myers Squibb, AstraZeneca, Boehringer Ingelheim, Eli Lilly, Takeda and Merck Sharp & Dohme.

Conflict of interest

References


