

The role of the kidneys in glucose homeostasis: a new path towards normalizing glycaemia

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The maintenance of normal glucose homeostasis requires a complex, highly integrated interaction among the liver, muscle, adipocytes, pancreas and neuroendocrine system. Recent studies have showed that the kidneys also play a central role in glucose homeostasis by reabsorbing all the filtered glucose, an adaptive mechanism that ensures sufficient energy is available during fasting periods. This mechanism becomes maladaptive in diabetes, however, as hyperglycaemia augments the expression and activity of the sodium–glucose cotransporter (SGLT) 2 in the proximal tubule of the kidney. As a result, glucose reabsorption may be increased by as much as 20% in individuals with poorly controlled diabetes. SGLT2 is a low-affinity, high-capacity glucose transport protein that reabsorbs 90% of filtered glucose, while the high-affinity, low-capacity SGLT1 transporter reabsorbs the remaining 10%. SGLT2 represents a novel target for the treatment of diabetes. In animal studies, SGLT2 inhibition reduces plasma glucose levels, resulting in improved β -cell function and enhanced insulin sensitivity in liver and muscle. Human studies have confirmed the efficacy of SGLT2 inhibitors in improving glucose control and reducing the A1c. Because the mechanism of SGLT2 inhibition is independent of circulating insulin levels or insulin sensitivity, these agents can be combined with all other antidiabetic classes, including exogenous insulin. Although the long-term efficacy and safety of SGLT2 inhibitors remain under study, the class represents a novel therapeutic approach with potential for the treatment of both type 2 and 1 diabetes.

Keywords: clinical trials, diabetes, glucose homeostasis, glycosuria, hyperglycaemia, kidneys, renal glucose transport, SGLT2, SGLT2 inhibitors, sodium–glucose cotransporters

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Introduction

Insulin resistance in muscle, liver and adipocytes and impaired insulin secretion are the core defects in type 2 diabetes [1–4]. Excess glucose production by the liver and decreased glucose utilization by insulin target tissues result in fasting and postprandial hyperglycaemia [1,5]. The β -cell dysfunction and insulin resistance can be detected long before the development of overt diabetes [2]. As shown in Table 1, at least eight metabolic or hormonal abnormalities contribute to the development of hyperglycaemia [4]. Most of these metabolic abnormalities have been well described elsewhere and will not be detailed further here [1–10].

Until recently, little attention has been focused on the role of the kidney in glucose homeostasis. However, along with the liver, this organ plays a vital role in ensuring that energy needs are met during fasting periods. Approximately 180 l of plasma per day are filtered by the kidney, which

works to maintain intravascular volume and acid-base, electrolyte and water balance by reabsorbing water, sodium, chloride and bicarbonate and secreting hydrogen ions and potassium produced by ingested foodstuffs. The kidney also plays a critical role in absorbing all of the filtered glucose. With a glomerular filtration rate of 180 l per day and a plasma glucose concentration of 5 mmol/l, the kidney filters approximately 162 g (900 mmol) of glucose per day, thereby helping to maintain normal fasting plasma glucose (FPG) levels (\sim 5.6 mmol/l) [11,12]. The kidney has developed a very efficient adaptive system involving the sodium–glucose cotransporter (SGLT) 2 and SGLT1 to reclaim all of the filtered glucose. When plasma glucose levels exceed the maximal reabsorptive capacity of the renal SGLT transport system, glycosuria occurs.

Expression and activity of SGLT2—the transport protein responsible for 80–90% of renal glucose reabsorption [13,14]—are increased in type 2 diabetes [15]. As a result, a higher than normal amount of glucose is reabsorbed by the kidneys into the bloodstream, thereby contributing to and maintaining hyperglycaemia. Chronically elevated plasma glucose levels exacerbate insulin resistance and β -cell dysfunction (i.e. glucotoxicity), further contributing to the abnormal glucose homeostasis that characterizes type 2 diabetes [2,4,12].

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Table 1. Pathogenic factors contributing to hyperglycaemia [4].

Tissue	Defect	Pathophysiologic effect
Pancreas— β -cells	Decreased insulin secretion	Fasting and postprandial hyperglycaemia
Pancreas— α -cells	Increased glucagon secretion	Excessive stimulation of hepatic glucose production
Liver	Insulin resistance Increased hepatic glucose output	Increased fasting and postprandial glucose
Muscle	Insulin resistance Decreased glucose uptake	Increased fasting and postprandial glucose
Gut	Decreased GLP-1/GIP secretion β -cell glucose resistance to GLP-1/GIP	Reduced postmeal insulin secretion
Adipose	Increased lipolysis	Increased plasma free fatty acids exacerbate insulin resistance in muscle and liver and impair β -cell function
Brain	Neurotransmitter dysfunction and insulin resistance	Impaired satiety signals and impaired neurohormone signalling
Kidney	Increased glucose reabsorption	Increased plasma glucose

GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1.

The complex pathophysiology and progressive nature of type 2 diabetes often render monotherapy ineffective with currently available agents [4,16–18]. Solving this problem becomes a more acute issue when considering the fact that, by year 2025, 380 million individuals worldwide are projected to have diabetes, with the prevalence more than doubling in many regions [19]. These management challenges leave a very large number of patients well above target glucose levels [19–21]. As showed by the recent 20-year follow-up of the United Kingdom Prospective Diabetes Study, improved control of blood glucose in newly diagnosed patients significantly decreases the long-term risk of both microvascular and macrovascular complications [22]. In contrast, three large trials of intensive glucose control in with diabetes with diabetes and a long duration of disease failed to show a macrovascular benefit [23–25]. The essential lesson to be learned from these trials is the importance of early therapeutic intervention to preserve β -cell function, increase insulin sensitivity and prevent micro- and macrovascular complications [26]. To accomplish these goals, we must continue to explore new therapeutic options as novel pathophysiologic mechanisms responsible for type 2 diabetes are elucidated.

Renal Glucose Transport

SGLT2 is a high-capacity, low-affinity glucose transporter (GLUT) located in the early convoluted segment (S1) of the proximal tubule, where luminal glucose is abundant [13,14,27]. The SGLT2 transporter mediates 90% of renal glucose

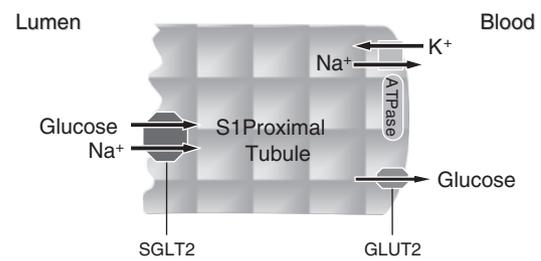


Figure 1. Glucose transport in the renal proximal tubule cell. Adapted with permission from Ref. [28]. ATPase, adenosine triphosphatase; GLUT, glucose transporter; SGLT, sodium–glucose cotransporter.

reabsorption by coupling glucose transport to the electrochemical sodium gradient (figure 1) [11–14]. First, sodium is absorbed across the luminal cell membrane, creating an energy gradient that permits glucose to passively enter the cell. Then, an adenosine triphosphatase (ATPase)-mediated sodium–potassium pump returns the sodium to the bloodstream. This exchange alters the concentration gradient within the cell, and glucose diffuses to the basolateral GLUT2, through which it passes back into the bloodstream [28].

The other 10% of renal glucose reabsorption occurs through SGLT1, a high-affinity, low-capacity transport protein that is found in the more distal, straight section of the proximal tubule (S3), where there is less luminal glucose [14,27]. SGLT1 also resides in the intestine, where it is responsible for absorption of dietary glucose and galactose [11,12]. Because SGLT1 resides in intestinal as well as renal tissues, and because it is not specific for glucose alone, it is not considered a viable target for therapeutic intervention. Inhibition of this transporter has the potential to cause osmotic diarrhoea and malabsorption. However, as long as clinically significant gastrointestinal side effects are not observed, combined SGLT2/SGLT1 inhibition remains a therapeutic option.

In the kidney, the amount of glucose reabsorbed through the SGLT1 and SGLT2 transporters is equal to the amount of glucose that is filtered by the glomerulus. Glucose reabsorption by the proximal tubule increases linearly with increasing glucose concentration, up to a theoretical threshold of approximately 11 mmol/l (figure 2). At this concentration, the glucose transport system becomes saturated and all the filtered glucose in excess of this threshold is excreted in the urine. This threshold varies from nephron to nephron, because of both anatomical and physiologic heterogeneity between nephrons, and this results in slight differences in glucose reabsorption levels between individual renal tubules. Thus, the actual threshold at which glucose starts to appear in the urine is slightly below the maximum of 11 mmol/l and occurs gradually in a curvilinear slope that begins at approximately 10 mmol/l. The difference between the actual and theoretical thresholds is known as ‘splay’ in the glucose titration curve [12]. The maximal transport rate for glucose (T_{mG}) varies among individuals, but it has an average value of approximately 375 mg/min for healthy subjects [29]. Like the glucose excretion threshold, the actual T_{mG} occurs, not at a precise cutpoint, but in a curvilinear manner that mirrors the excretion threshold [12].

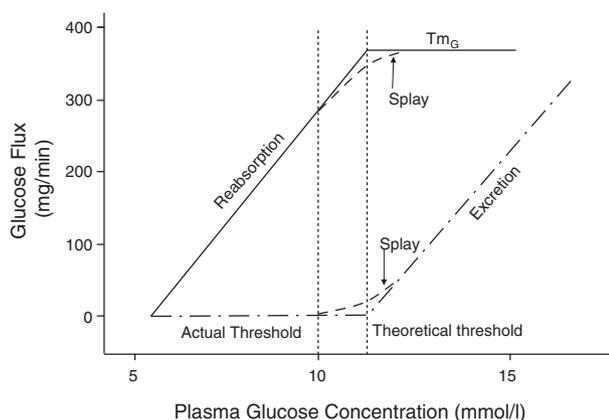


Figure 2. Renal glucose handling. Adapted with permission from Ref. [12]. T_{mG} , maximal transport rate for glucose.

Defective Renal Glucose Reabsorption: An Emerging Treatment Target in Type 2 Diabetes

Experimental evidence in rodents and emerging data in humans indicate that the renal reabsorptive threshold for glucose is increased in patients with diabetes. In both type 2 [30] and type 1 diabetes [31], the T_{mG} is increased by approximately 20%. Similarly, in animal models of both type 2 and 1 diabetes, the rate of glucose reabsorption and GLUT activity is increased. GLUT2 expression and activity were significantly increased in Zucker diabetic fatty (ZDF) rats compared with controls, although there was no difference in SGLT2 expression between the groups [27,32,33]. In cultured human renal proximal epithelial cells from the urine of patients with type 2 diabetes, SGLT2 and GLUT2 mRNA and protein expression are markedly increased compared with normal glucose-tolerant controls (figure 3). Renal glucose uptake, measured with methyl- α -D-[U- 14 C]-glucopyranoside [a non-metabolizable glucose analogue (AMG)], also was significantly increased in diabetic proximal tubular cells [15]. These results

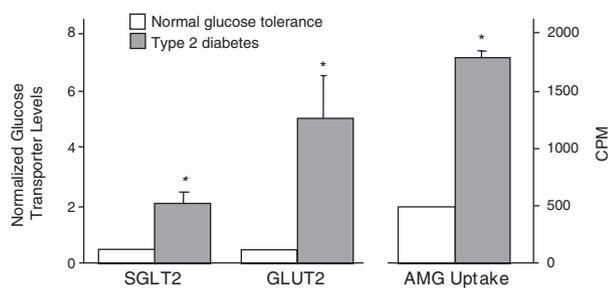


Figure 3. SGLT2 and GLUT2 protein expression and AMG uptake in cultured renal proximal tubular epithelial cells from patients with type 2 diabetes and control subjects with normal glucose tolerance. Adapted with permission from Ref. [15]. AMG, methyl- α -D-[U- 14 C]-glucopyranoside; GLUT, glucose transporter; SGLT, sodium-glucose cotransporter. Copyright 2005, American Diabetes Association. From Diabetes®, Vol. 54, 2005; 3427–3434. Reprinted with permission from The American Diabetes Association.

in rodents and humans suggest that chronic hyperglycaemia upregulates SGLT2/GLUT2 transport expression and activity.

During evolution, the kidney developed an intricate system to reabsorb all the filtered glucose to conserve energy at a time when energy intake was sparse. From an evolutionary standpoint, the increase in SGLT2 transport in response to hyperglycaemia can be viewed as an adaptive response. However, in the patient with diabetes this adaptive response becomes maladaptive, and glycosuria is not observed until the plasma glucose concentration increases to levels that are substantially higher than 11 mmol/l—the glucose concentration threshold in non-diabetic individuals. Thus, instead of allowing the kidney to excrete the excess filtered glucose in the urine and correct the hyperglycaemia, the SGLT2 transporter works counterproductively to maintain the elevated plasma glucose concentration [12].

Inhibition of SGLT2 transport ‘resets’ the system by lowering the threshold for glycosuria (figure 4), leading to correction of the hyperglycaemia. Normalization of the blood glucose level ameliorates insulin resistance in muscle by augmenting insulin signalling, GLUT4 and glycogen synthase activity [34,35]. In the liver, correction of hyperglycaemia decreases glucose-6-phosphatase and PEP carboxykinase activity, leading to a decrease in gluconeogenesis and total hepatic glucose production (HGP), with a resulting decrease in FPG concentration [36,37]. Correction of the hyperglycaemia also improves β -cell function [34,38]. Collectively, the deleterious effects of chronic hyperglycaemia on β -cell function and on liver and muscle insulin sensitivity are referred to as ‘glucotoxicity’ [34,39].

Phlorizin Studies in Experimental Models of Type 2 Diabetes

Initial evidence that reversing glucotoxicity by augmenting renal glucose excretion could lead to improved glycaemic control has come from animal studies using phlorizin, a molecule that inhibits both the SGLT1 and SGLT2 transporters

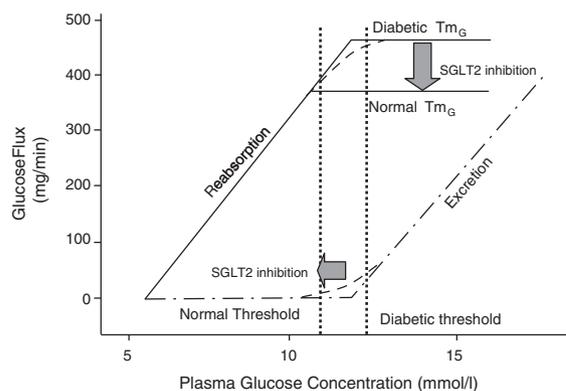


Figure 4. Effect of SGLT2 inhibition on renal glucose handling in diabetes. SGLT2 inhibitors reduce the T_{mG} for glucose reabsorption, thereby lowering the glucose excretion threshold and bringing the glucose reabsorption threshold closer to normal (grey arrows). Adapted with permission from Refs [12,31]. SGLT, sodium-glucose cotransporter; T_{mG} , maximal transport rate for glucose.

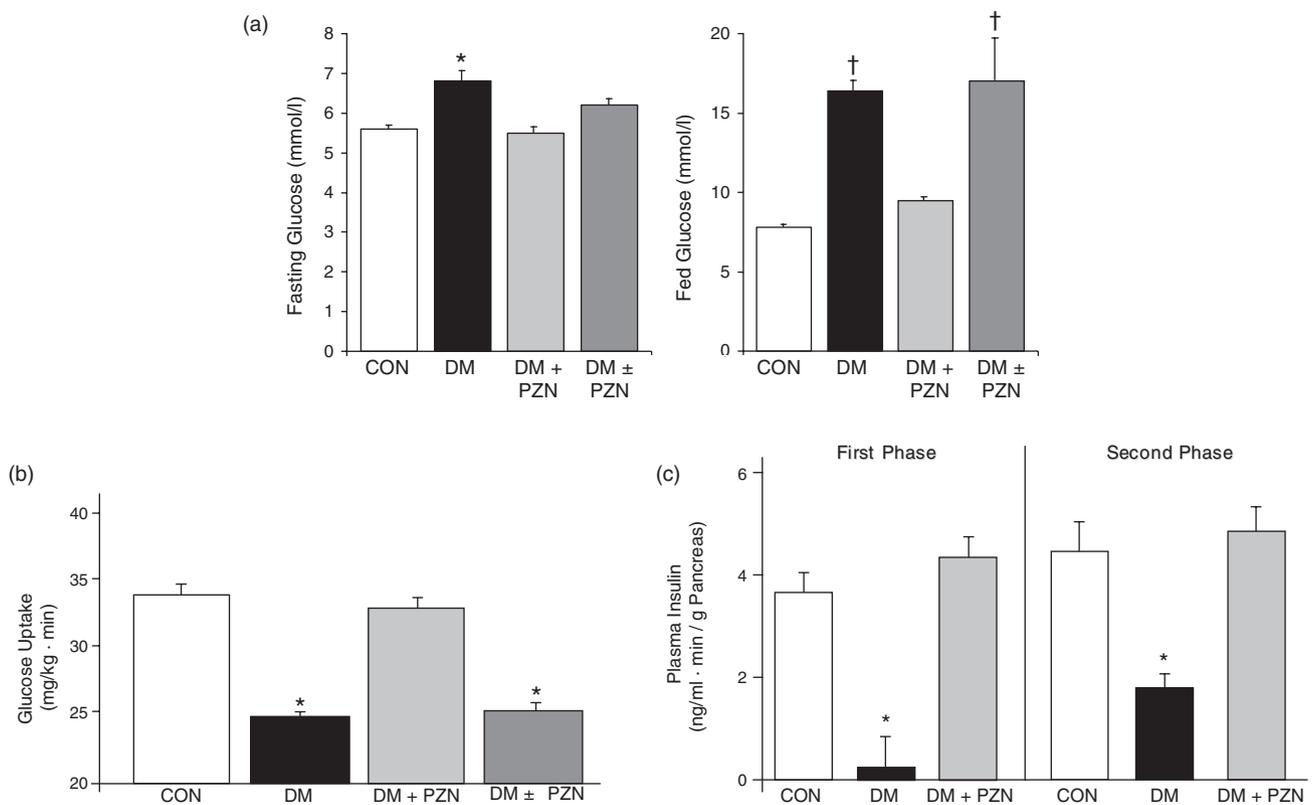


Figure 5. Effects of phlorizin treatment on plasma glucose concentration, insulin sensitivity and β -cell function in diabetic rats. CON, control; DM, diabetes (90% pancreatectomy), no treatment; DM + PZN, diabetes, phlorizin treatment for 4–5 weeks; DM \pm PZN, diabetes, phlorizin treatment for 4–5 weeks, followed by phlorizin discontinuation for 10–12 days, after which time animals were studied. (a) Fed and fasting plasma glucose. * $p < 0.05$, † $p < 0.001$ vs. control and phlorizin treatment. Adapted with permission from Ref. [40]. (b) Insulin-mediated glucose uptake. * $p < 0.001$ vs. control and phlorizin treatment. Adapted with permission from Ref. [40]. (c) First- and second-phase insulin response. * $p < 0.001$ vs. control. Adapted with permission from Ref. [42].

(and therefore has not been studied for use in humans). In partially pancreatectomized diabetic rats, phlorizin normalized both fasting and postprandial plasma glucose concentrations. Withdrawal of phlorizin was associated with a return to the diabetic state (figure 5a) [40]. In the same study, correction of hyperglycaemia with phlorizin resulted in a marked improvement in peripheral insulin sensitivity from 24.8 ± 0.6 to 33.1 ± 1.1 mg/kg min ($p < 0.001$; figure 5b) [40]. In another study in rats with streptozotocin-induced diabetes, phlorizin treatment restored glucose utilization to normal levels [41]. Improved first- and second-phase insulin secretion also has been shown after correction of the hyperglycaemia with phlorizin in diabetic rats (figure 5c) [42]. Discontinuation of phlorizin led to a return of insulin resistance and a decline in β -cell function [40,42]. Correction of hyperglycaemia with phlorizin in diabetic dogs has also been shown to normalize the elevated plasma glucagon levels that are associated with the diabetic state [43].

Similar improvements in β -cell function and insulin sensitivity have been observed with T-1095, an oral phlorizin derivative that no longer is in clinical development [37,44,45]. In streptozotocin-induced diabetic rats, both insulin-stimulated glucose disposal and the elevated basal rate of HGP were

normalized, and insulin signalling in skeletal muscle and liver was enhanced [44].

Animal Studies with Agents in Clinical Development

Phlorizin must be administered by injection and inhibits both SGLT1 and SGLT2, so it has served primarily as a research tool. However, results obtained with phlorizin have provided the scientific basis for the development of specific SGLT2 inhibitors for the treatment of type 2 diabetes (Table 2). Dapagliflozin is the most advanced of these inhibitors in clinical development. In both normal and ZDF rats, dapagliflozin in doses ranging from 0.1 to 10 mg/kg body weight markedly increased renal glucose excretion and significantly decreased FPG by day 15 [46]. No change in FPG was observed in normal rats treated with dapagliflozin because of a compensatory increase in HGP that maintained normoglycaemia. In contrast, HGP decreased significantly in the dapagliflozin-treated vs. untreated ZDF rats. Whole-body insulin-stimulated glucose disposal increased significantly with dapagliflozin treatment [46]. ZDF rats fed a high-fat diet and treated with dapagliflozin also had improved pancreatic function and improved insulin sensitivity [47].

Similar results have been seen with other SGLT2 inhibitors in development. Canagliflozin (JNJ-28431754) lowers blood

Table 2. SGLT2 inhibitors in clinical development.

Clinical development phase	Agent	Manufacturer
Phase III	Dapagliflozin	AstraZeneca/Bristol-Myers Squibb
	BI 10773	Boehringer Ingelheim
	Canagliflozin (JNJ-28431754)	Johnson & Johnson
Phase II	AVE-2268	Sanofi-Aventis
	Remogliflozin (discontinued)	Glaxo SmithKline/Kissei
	Sergliflozin (discontinued)	
	TS-033	Taisho
	YM-543	Astellas/Kotobuki Pharmaceuticals
Phase I	ISIS 388626	ISIS
	CSG-452A	Chugai/Roche
	SAR-7226	Sanofi-Aventis
	TA-7284	Mitsubishi Tanabe/Johnson & Johnson

SGLT, sodium–glucose cotransporter.

glucose levels and decreases body weight in obese diabetic animals [48]. Finally, when compared with remogliflozin (which recently was discontinued) in a study of mice and rats, BI 10773 had more potent inhibition of SGLT2 and produced significantly greater 24-h urinary glucose excretion [49].

Preclinical data also support the potential of ISIS 388626, an SGLT2 antisense oligonucleotide that is highly specific for the renal SGLT2 transporter. In rats and dogs, this compound decreased the SGLT2 mRNA and protein by approximately 80% without any effect on SGLT1. FPG, postprandial glucose and glycated haemoglobin (HbA1c) were reduced significantly with ISIS 388626, without changes in plasma or urine electrolytes [50]. Treatment of normal cynomolgus monkeys increased glycosuria >1000-fold without inducing hypoglycaemia [51].

Clinical Evidence for SGLT2 Inhibition as Therapy for Type 2 Diabetes

On cursory review, a diabetes treatment strategy that increases glycosuria may seem counter intuitive. However, in addition to animal data supporting the effectiveness of this approach, a human genetic model has shown its long-term safety. Familial renal glycosuria (FRG) results from a mutation in the gene for SGLT2; 21 different mutations in 21 different families have been described [52,53]. While individuals with type A FRG have reduced levels of an abnormal SGLT2 protein, which results in a lower T_{mG} , individuals with type B FRG are characterized by SGLT2 transporters with a diminished affinity for glucose, resulting in an exaggerated splay but a normal T_{mG} [53]. Regardless of FRG type, affected individuals excrete as much as 100 g of glucose per day in their urine but nevertheless remain asymptomatic [11,52]. Blood glucose concentrations remain normal because of an increased rate of HGP, which

precisely counterbalances the amount of glucose that is lost in the urine. Plasma volume and electrolyte composition remain normal because fluid and electrolytes that are not absorbed in the proximal tubule are completely reabsorbed in more distal parts of the nephron. These individuals have normal kidney and bladder function and no increased incidence of diabetes or urinary tract infection [11,12,52,53].

The benign nature of FRG has established SGLT2 inhibition as a feasible approach to the treatment of patients with diabetes. To date, the majority of available human trial reports on the safety and efficacy of SGLT2 inhibitors have not revealed major adverse side effects. The most commonly encountered side effect has been fungal infection of the genital organs. An increased incidence of bacterial urinary tract infections also has been described in some studies (see subsequent discussion).

Dapagliflozin is the SGLT2 inhibitor most advanced in clinical development; in humans [54] it is rapidly absorbed with maximum plasma concentrations (C_{max}) observed within 2 h and half-life of ~17 h [54]. Dapagliflozin is highly protein bound (97–98%) and renal excretion is low (2–4%) [54]. Approximately 0.1% of dapagliflozin is excreted as an inactive metabolite [54], which has a bioavailability of 84%, with a half-life of 4.6 h. In humans, the free dapagliflozin fraction is 4% at a 10 μ M plasma concentration. In Chinese hamster ovary cells expressing both SGLT1 and SGLT2, dapagliflozin has a 1200-fold greater selectivity for SGLT2 vs. SGLT1, with a K_i of 1.1 nM for SGLT2 and 1390 nM for SGLT1 [55].

In a 12-week, randomized, double-blind, placebo-controlled study involving 389 treatment-naïve patients with type 2 diabetes, dapagliflozin significantly reduced HbA1c by 0.4–0.7% across doses ranging from 2.5 to 50 mg ($p < 0.01$ vs. placebo; figure 6) [56]. The active comparator, metformin extended release 1500 mg. Glycosuria increased in a dose-dependent fashion by 52 g (289 mmol)/day to 85 g (472 mmol)/day in the dapagliflozin groups. This was associated with a dose-related decrease in FPG, ranging from 1.1 to 1.7 mM, vs. a 1.0 mM decrease with metformin. Dapagliflozin caused a dose-related decline in the mean 3-h postprandial glucose area under the curve from 12,215 to 8,913 mg/min/dl compared with 7775 mg/min/dl for metformin [56]. HbA1c reductions were numerically similar between metformin and the 2.5 and 5 mg doses of dapagliflozin, while dapagliflozin 10 and 50 mg yielded slightly greater decreases in HbA1c (figure 6) [56]. In a large ($n = 546$) 24-week trial in metformin-treated patients with type 2 diabetes, dapagliflozin in doses of 2.5, 5 and 10 mg/day reduced the HbA1c by –0.67, –0.70 and –0.84%, respectively, compared to placebo (–0.3%) (all $p < 0.01$) [57]. Body weight was reduced –0.3% by 2.26, 3.10 and 2.96 kg, respectively, compared to controls (–0.87 kg) (all $p < 0.01$) [57]. In 485 patients with type 2 diabetes who were controlled by diet and exercise, dapagliflozin in doses of 2.5, 5.0 and 10 mg/day reduced the HbA1c by –0.58, –0.77 and –0.89% and body weight by –3.3, –2.8 and –3.2 kg after 24 weeks [58]. In a subgroup of 74 patients with diabetes with HbA1c = 10.1–12.0% (87–108 mmol/mol), 24 weeks of dapagliflozin treatment at doses of 5 and 10 mg/day reduced the HbA1c by 2.88% (8 mmol/mol) and 2.66% (6 mmol/mol)

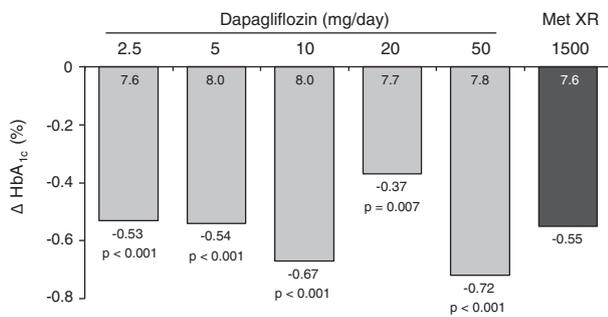


Figure 6. Placebo-adjusted HbA1c reduction after 12 weeks of treatment with dapagliflozin or extended release metformin. Baseline HbA1c values are indicated at the top of each bar. p values indicate comparison with placebo. No statistical comparisons with metformin were made. Adapted with permission from Ref. [56].

[58]. In a preliminary study, Nauck et al. [59] compared the efficacy of dapagliflozin (n = 400) and glipizide (n = 401) in metformin-treated patients with T2DM and a starting HbA1c of 7.7% (61 mmol/mol). After 52 weeks, the decrement in the HbA1c was identical (−0.52%) in both treatment groups [59]. Dapagliflozin-treated subjects lost on average 3.2 kg, while glipizide-treated subjects gained 1.4 kg (p < 0.0001). Both systolic (−4.3 vs. +0.8 mmHg, p < 0.001) and diastolic (−1.6 vs. −0.4 mmHg, p = 0.02) blood pressure declined more with dapagliflozin. In a provocative study, Wilding et al. [60] randomized 71 insulin-treated (~50 units/day) patients with type 2 diabetes who also were receiving an insulin sensitizer (metformin and/or thiazolidinedione) to add on therapy with dapagliflozin (5 and 10 mg/day) or placebo. Although the insulin dose was reduced by 50% at the start of therapy (the insulin sensitizer dose was unchanged), after 12 weeks of dapagliflozin therapy, the HbA1c declined by 0.70–0.78% (p < 0.01 vs. placebo). The placebo-subtracted reductions in body weight were 2.6 and 2.4 kg, respectively (p < 0.01 vs. placebo). Both the increase in glycosuria and 50% reduction in insulin dose could have contributed to the weight loss in dapagliflozin-treated subjects. Zhang et al. [61] compared 151 early stage (diabetes duration = 1 year) and 58 late stage (diabetes duration = 11 years) patients with type 2 diabetes randomly assigned to receive 10 or 20 mg/day of dapagliflozin for 12 weeks [61]. The late stage diabetic group had a HbA1c = 8.4% (69 mmol/mol) and was on large dose of insulin (>50 units/day) plus metformin and a thiazolidinedione, and had long standing diabetes (mean = 11.1 years) compared to the early stage group (diabetes duration = 1.0 years, HbA1c = 7.6% (60 mmol/mol), no antidiabetic medications) [61]. The decline in HbA1c (0.5–0.7 vs. 0.6–0.8%, respectively) was similar in late and early stage patients with diabetes [61]. This is explained by the unique mechanism of action of dapagliflozin on the kidney that is independent of the severity of insulin resistance or β-cell failure. A greater reduction in body weight was observed in the late stage diabetic group and this most probably is explained by the reduction in insulin dose since glucose excretion was similar in both groups.

In a 12-week study involving 71 patients with poorly controlled glycaemia despite therapy with insulin plus

metformin and/or a thiazolidinedione, the addition of dapagliflozin 10 and 20 mg significantly reduced HbA1c by 0.61% [confidence interval (95% CI) 0.9%, 0.4%] and 0.69% (CI 0.9%, 0.4%), respectively, compared with a 0.09% (CI 0.2%, 0.4%) reduction in the placebo group. Significant decreases in FPG and postprandial glucose were also observed in this study [62].

There are a number of other SGLT2 inhibitors currently under development or in clinical trials. In a phase 1 study, a single dose of sergliflozin (50–500 mg) caused a dose-dependent increase in glycosuria in both normal subjects and patients with T2DM [63,64]. The 500 mg dose reduced the mean plasma glucose concentration during the OGTT from 18.3 to 11.2 mM [63]. More prolonged treatment (14 days) with sergliflozin also induced dose-dependent glycosuria with modest weight loss [64].

In a double-blind, placebo-controlled, dose-ranging study in 451 metformin-treated T2DM subjects canagliflozin in doses of 50, 100, 200 and 300 mg/day for 12 weeks reduced the HbA1c by 0.7–0.9% from baseline and 0.5–0.7% vs. placebo in association with weight loss of 1.3–2.3% [65]. In a 16 day trial, canagliflozin improved β-cell function in patients with type 2 diabetes using a model-based method to calculate insulin secretion [66]. In a small study involving 29 subjects with T2DM who were sub-optimally controlled (HbA1c = 8.4%) (69 mmol/mol) with insulin, canagliflozin at 100 and 300 mg/day for 28 days reduced the HbA1c by 0.7 and 0.9%, respectively [67]. In a single dose study, BI10773 in doses ranging from 1 to 100 mg caused a dose-dependent increase in urine glucose excretion in healthy male subjects [68]. At the 100 mg dose, BI10773 increased urinary glucose excretion to 74 g over 24 h and reduced the plasma glucose excursion during an OGTT. In a 12-week double-blind study, 361 Japanese patients with type 2 diabetes who were treated with ASP1941 at doses ranging from 12.5 to 100 mg/day experienced a 0.9% reduction in HbA1c at the two highest doses (50 and 100 mg/day) [69]. Body weight also was dose-dependently reduced by up to 2 kg in the 100 mg/day dose. In a Phase 2A study, LX4211, which inhibits SGLT2 and to a lesser extent SGLT1, at doses of 150 and 300 mg/day reduced the HbA1c by 1.2% but the starting HbA1c (8.2–8.5%) (66–70 mmol/mol) was higher than in most other studies and the placebo decreased the HbA1c by 0.5% [70]. Sanofi-Aventis recently has initiated human trials with AVE2268 [71]. In mice and rats this compound was shown to be highly selective for SGLT2 and caused a significant dose-dependent increase in urinary glucose excretion and reduction in blood glucose concentration during an OGTT [71]. Remogliflozin, which was developed by Kissei Pharmaceuticals and GlaxoSmithKline, has been discontinued, apparently to make way for development of the SGLT2 inhibitor (KGA-3235).

Because glycosuria translates into a loss of calories through the urine, SGLT2 inhibition would be expected to cause weight loss, and this has been borne out in clinical trials with dapagliflozin. When measured, dapagliflozin causes the urinary loss of 60–80 g of glucose per day, which equates to 240–320 cal/day or 2–3 pounds per month if this deficit is not offset by increased caloric intake. Consistent with this, after 12

Table 3. Selected adverse events after 12 weeks of treatment with dapagliflozin vs. placebo and metformin [50].

Event	Dapagliflozin dose		Placebo		Metformin	
	2.5 mg	5 mg	10 mg	20 mg	50 mg	
Urinary tract infection*	3 (5)	5 (9)	5 (11)	7 (12)	5 (9)	3 (6) 5 (9)
Genital infection†	2 (3)	1 (2)	1 (2)	4 (7)	4 (7)	0 (0) 1 (2)
Hypotension	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	1 (2) 2 (4)
Hypoglycaemia‡	4 (7)	6 (10)	3 (6)	4 (7)	4 (7)	2 (4) 5 (9)
Change in urine output (ml/24 h)	107	340§	375§	375§	470§	-112 -96
Change in creatinine (mg/dl)	-0.01	0.0	-0.02	-0.01	0.02	0.0 -0.02
Change in BUN (mg/dl)	1.07§	0.71§	2.03§	0.87§	1.32§	-0.96 -0.18
Change in Mg ⁺⁺ (mEq/l)	0.07	0.10	0.12§	0.14§	0.18§	0.04 -0.03
Change in uric acid (mg/dl)	-1.03§	-1.12§	-0.98§	-1.13§	-1.14§	-0.16 0.18
Hematocrit (vol %)	1.51§	2.03§	1.95§	2.57§	2.86§	-0.08 -1.12

The numbers in parentheses indicate the percent. BUN, blood urea nitrogen; Mg⁺⁺, magnesium.

*Includes cystitis and bacterial and fungal urinary tract infection.

†Includes vulva vaginal mycotic infection, genital herpes, genital infection and penile infection.

‡Hypoglycaemia was not defined, but there was no reported fingerstick glucose value ≤ 2.8 mmol/l.

§p < 0.05 vs. placebo.

weeks of dapagliflozin treatment among drug-naïve patients, mean body weight decreased by 2.5–3.4 kg, compared with a 1.7 kg loss in the metformin group [56]. Similar weight loss has been observed in other dapagliflozin studies [57–59,61]. In the study of insulin-treated patients, dapagliflozin caused a weight loss of 4.3–4.5 kg vs. 1.9 kg among patients receiving placebo [60].

The long-term (beyond 12 months) durability of dapagliflozin's effect on glycaemic control and weight loss remains to be determined. It also is possible that after plasma glucose levels return to the normal range, the effectiveness of SGLT2 inhibition may wane. However, as these agents cause persistent glycosuria in individuals with normal glucose tolerance, it is reasonable to assume that they will maintain their glycaemic efficacy in patients with type 2 diabetes, especially when coupled with agents such as metformin or incretin-based therapies, which reduce HGP.

It is noteworthy that the increase in urine glucose excretion (60–80 g/day) with all SGLT2 inhibitors represents <50% of the filtered glucose load. The failure to observe a greater inhibition of renal glucose reabsorption could be explained by (i) inability of the SGLT2 inhibitor to interact with the SGLT2 transporter because of their anatomical location; (ii) competitive inhibition, which progressively raises the local glucose concentration at the site of the SGLT2 transporter, thus reducing its effectiveness; (iii) insufficiently high drug concentrations in the tubular lumen to inhibit the SGLT2 inhibitor; (iv) in man, GLUTs other SGLT2 are responsible for a much greater fraction of glucose reabsorption than previously reported; (v) up-regulation of SGLT1 or other GLUTs. The later seems unlikely since the magnitude of glycosuria on days 1–3 vs. day 14 after the start of dapagliflozin is similar [56].

Safety of SGLT2 Inhibitors

No long-term safety data are available for the SGLT2 inhibitors. Possible safety/tolerability considerations include

the risk of urogenital infection, electrolyte imbalance, nocturia, intravascular volume depletion and nephrotoxicity as a result of accumulation of advanced glycation end products within the kidney. An increased incidence of vulva-vaginal infections in women and balanitis in males (~8–10% with dapagliflozin vs 3–5% in subjects receiving placebo) has been observed [56–63]. In some clinical studies, a small increase (3–5%) in the rate of urinary tract infections also has been reported. The majority of these infections involved the lower urinary tract, that is, cystitis, and responded to standard therapy [59–61]. Other side effects have not been observed with dapagliflozin (Table 3), and the long-term follow-up of individuals with FRG indicates that such side effects are unlikely to be encountered with the SGLT2 inhibitors as a result of the glycosuria per se. However, this does not exclude the possibility that the molecule used to induce glycosuria or that the combination of hyperglycaemia plus glycosuria might be injurious to the kidney. In a 12-week trial with dapagliflozin, serum magnesium increased slightly but significantly, while the serum uric acid declined by approximately 1 mg/dl. There were no clinically relevant changes in serum sodium, calcium, phosphate or potassium levels [56]. It is possible that the mechanism of action of these agents may limit their use in patients with renal impairment. If the glomerular filtration rate is significantly reduced, this would be expected to reduce the filtered glucose load and diminish their glycaemic effect.

In a 12-week trial, small increases in blood urea nitrogen (BUN) and haematocrit have been observed in dapagliflozin-treated patients [56,57], but serum creatinine did not change and the change in BUN-creatinine ratio was not dose-dependent. No hypotension has been observed, although dapagliflozin did yield decreases in systolic blood pressure of 3–7 mmHg, a potentially beneficial result that warrants further study [56–59]. Hypoglycaemia has not been observed with the SGLT2 inhibitors [56–59].

Although, to date, there is no evidence that the SGLT2 inhibitors are associated with deterioration in renal function, all published studies are of relatively short duration

(6–12 months). To the contrary and speculative at present, it is possible that SGLT2 inhibitor therapy may prevent diabetic nephropathy. First, improved glycaemic control reduces the risk of diabetic complications [72,73]. Second, by enhancing sodium delivery to the juxtaglomerular apparatus, SGLT2 inhibition might have a renal protective effect, independent of glucose reduction. In type 2 diabetes, increased glucose and sodium absorption in the proximal tubule reduces the amount of sodium available for delivery to the juxtaglomerular apparatus. As a result, the glomerulo-tubular feedback reflex is activated, leading to increased renal plasma flow, elevated intra-glomerular pressure and increased glomerular filtration rate. Together, these restore normal salt delivery to the juxtaglomerular apparatus, but at the expense of increased intra-glomerular pressure. These changes in renal haemodynamics lead to renal hypertrophy and eventually to the development of diabetic nephropathy [74–76]. Normalization of the plasma glucose concentration with insulin reduces the filtered glucose load and has been shown to reverse renal hyperfiltration and reduce kidney size [76]. SGLT2 inhibitors may prevent diabetic nephropathy not only by reducing the plasma glucose concentration and therefore the filtered glucose load but also by increasing sodium delivery to the distal nephron, thereby inhibiting the glomerulo-tubular feedback reflex.

Summary

Until recently, excessive renal glucose reabsorption has not been considered a pathophysiological derangement that contributes to the development of hyperglycaemia in individuals with diabetes. By reducing glycosuria, enhanced proximal tubular glucose reabsorption helps maintain hyperglycaemia, thereby contributing to insulin resistance in both liver and muscle and impairing insulin secretion, the core defects of type 2 diabetes. By correcting hyperglycaemia and reducing glucotoxicity, SGLT2 inhibitors may have a disease-modifying effect.

The pathogenesis of type 2 diabetes involves numerous defects in a wide variety of tissues. No single antidiabetic agent can correct all these metabolic disturbances, and effective antidiabetic therapy will require multiple drugs used in combination. With a unique mechanism of action—increased urinary glucose excretion—the SGLT2 inhibitors can be used as monotherapy as well as in combination with currently available antidiabetic agents. The SGLT2 inhibitors carry little or no risk of hypoglycaemia because they do not affect glucose counter regulatory mechanisms. In fact, because the action of SGLT2 inhibitors is independent of insulin, this class has the potential to be combined with exogenous insulin as adjunctive therapy for type 1 diabetes—although combining a glycosuric agent with a fixed dose of insulin would be associated with a potential for hypoglycaemia. Because increased glycosuria results in caloric loss, these glycosuric agents can be expected to yield weight loss along with a reduction in plasma glucose levels. With these properties, the SGLT2 inhibitors have potential for use throughout the continuum of diabetes treatment.

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Conflict of Interest

Drs R. A. D. and S. D. P. are on the Advisory Board of Boehringer Ingelheim. Dr S. D. P. also serves as a consultant for Bristol-Myers Squibb and Astra Zeneca. Dr R. A. D. serves as a consultant for Bristol-Myers Squibb. Dr J. A. D. has no conflict of interest with regard to companies producing SGLT2 inhibitors. R. A. D., S. D. P. and J. A. D. designed this study, conducted data collection, performed the analysis, and wrote the article.

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