Renal sodium–glucose transport: role in diabetes mellitus and potential clinical implications

George L. Bakris[1], Vivian A. Fonseca[2], Kumar Sharma[3] and Ernest M. Wright[4]

[1]Hypertensive Diseases Unit, Department of Medicine, Pritzker School of Medicine, University of Chicago, Chicago, IL, USA; [2]Department of Medicine and Pharmacology, Tulane University Health Sciences Center, New Orleans, Los Angeles, USA; [3]Department of Medicine, University of California at San Diego/VA Medical System, La Jolla, California, USA and [4]Department of Physiology, Mellinkoff Professor in Medicine, Department of Physiology, David Geffen School of Medicine at UCLA, Los Angeles, California, USA

The kidneys play a major role in the regulation of glucose in humans, reabsorbing 99% of the plasma glucose that filters through the renal glomeruli tubules. The glucose transporter, SGLT2, which is found primarily in the S1 segment of the proximal renal tubule, is essential to this process, accounting for 90% of the glucose reabsorption in the kidney. Evidence has suggested that selective inhibition of SGLT2 induces glucosuria in a dose-dependent manner and may have beneficial effects on glucose regulation in individuals with type II diabetes. Preclinical data with SGLT2 inhibitors, such as dapagliflozin and sergliflozin, show that these compounds are highly selective inhibitors for SGLT2, have beneficial effects on the glucose utilization rate, and reduce hyperglycemia while having no hypoglycemic adverse effects. Clinical research remains to be carried out on the long-term effects of glucosuria and other potential effects of this class of drug. Nonetheless, these compounds represent a very promising approach for the treatment of diabetes.

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Correspondence: *George L. Bakris, Hypertensive Diseases Unit, Department of Medicine, Pritzker School of Medicine, University of Chicago, Chicago, Illinois 46321, USA. E-mail: gbakris@gmail.com*

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The kidney plays a pivotal role in the regulation of glucose reabsorption, and by extension has a major part in maintaining the overall metabolic balance of the body. In healthy humans, more than 99% of the plasma glucose that filters through the renal glomerulus is reabsorbed. Transport of glucose across cell membranes is accomplished by two gene families: the facilitative glucose transporters – the GLUTs – and by an active sodium-dependent transport process mediated by the sodium–glucose co-transporters (SGLTs).[1] The latter process allows glucose to be accumulated in cells against the concentration gradient.

The SGLTs are a large family of membrane proteins involved in the transport of glucose, amino acids, vitamins, osmolytes, and some ions across the brush-border membrane of the intestinal epithelium and of the proximal renal tubules.[1] The low-capacity, high-affinity Na/glucose co-transporter, SGLT1, is mainly expressed in the gastrointestinal tract, whereas the high-capacity, low-affinity transporter, SGLT2, is expressed primarily in the kidney.[1] In the intestine, SGLT1 performs an important function in the absorption of glucose.[2] SGLT3 is widely expressed throughout the body and can be found in the skeletal muscle and the enteric nervous system; however, it is not a glucose transporter, but appears to have a role as a glucose sensor.[2] Although there are other members of the SGLT family, including SGLT4, 5, and 6, little is known about the function of these isoforms in humans.[2,3]

SGLT2 AND GLUCOSE REABSORPTION IN THE PROXIMAL RENAL TUBULE

Although SGLT1 plays a primary role in absorption of glucose in the intestine, in the kidney SGLT2 is the primary factor. Accounting for 90% of glucose reabsorption in the kidney, SGLT2 is found primarily in the brush-border membrane of the S1 segment of the proximal renal tubule. SGLT1 is located more distally in the S3 segment of the proximal tubule and accounts for only 10% of renal glucose reabsorption.[4] Two basolateral membrane glucose transporters facilitate transcellular glucose transport – the low-affinity GLUT2, which works in conjunction with SGLT2 in the S1 segment of the renal tubule, and the high-affinity GLUT1, which works with SGLT1 in the S3 segment.[5]

Although the filtered load of glucose is ~180 g/day, in the normal kidney only ~500 mg of glucose is excreted in the urine over a 24-h period. The remaining is reabsorbed, as it flows from the glomerulus through the proximal tubule, passing from S1 to S3, where first SGLT2 and then SGLT1 transport glucose from the glomerular filtrate in the tubule lumen into the tubule cells. Once glucose enters the cells, GLUT2 and GLUT1 facilitate exit from the cell across the basolateral membrane into the interstitial fluid,[6] and from there it is picked up by the peritubular capillary. The efficiency of the transport system is such that as the filtrate moves from S1 to S2 and to S3, the glucose concentration decreases dramatically, and only a few millimeters away from the glomerulus, all of the glucose gets reabsorbed into the bloodstream. The thermodynamic machinery of the SGLTs couples glucose and sodium transport into the proximal tubule epithelial cells (Figure 1).

Gene mutation of SGLT1 has serious consequences for afflicted individuals, who experience glucose–galactose malabsorption, frequent watery diarrhea, and dehydration.[7] However, these individuals have little or no glucosuria. Approximately 21 different gene mutations have been described for SGLT2.[8] Those who have SGLT2 mutations have persistent renal glucosuria, with glucose excretion of up to 160 g/day. Although no other complications are known to be associated with mutations of this gene, it is a rare benign genetic condition not well studied.[8] The observation that in contrast to SGLT2 mutations, SGLT1 mutations are accompanied by little or no glucosuria supports a limited role for SGLT1 in the kidney and a much larger one for SGLT2.[7]

Phlorizin, a nonselective SGLT inhibitor, has been used to explore the role of SGLT activity in glucose regulation. However, it has significant limitations, both as an investigative tool and as a potential pharmacological treatment for diabetes. First, it is a potent inhibitor of both SGLT1 and SGLT2,[9] and thus it is not possible to use phlorizin to distinguish SGLT1 and SGLT2 activities. Second, oral phlorizin causes malabsorption of glucose and diarrhea (analogous to the inherited condition due to SGLT mutations). The development of the selective inhibitors of SGLT2, for example, sergliflozin and dapagliflozin, has helped elucidate the role of this transporter in the kidney.

SGLT2 AND DIABETES MELLITUS

There is a transport maximum (T_m) for glucose; in hyperglycemic individuals, when glucose levels approach 400 mg glucose per 100 ml plasma, the T_m for glucose of 375 mg/min is exceeded. Consequently, the patient begins to excrete large amounts of glucose in the urine even though the renal tubules continue to function normally.

The issue of gene expression and the possibility of SGLT adaptation to chronic hyperglycemia is an area rich for investigation. Studies in animal models of diabetes show that small amounts of adaptation do occur, and there appears to be a twofold increase in the expression of SGLT2 mRNA. The results of a recent study in rats with experimental diabetes[10] suggest that plasma glucose concentration is an important regulator of hepatocyte nuclear factor-1α transcription factor expression and activity.[10] Hepatocyte nuclear factor-1α directly controls SGLT2 gene expression in humans and rats.[10] Induction of diabetes in rats increased mRNA expression of both SGLT2 and hepatocyte nuclear factor-1α in the renal cortex. A 6-day treatment with insulin or with phlorizin improved glycemic control and reduced the expression of SGLT2 and hepatocyte nuclear factor-1α to near normal levels.[10] The authors of this study concluded that these findings suggest that enhanced SGLT2 expression

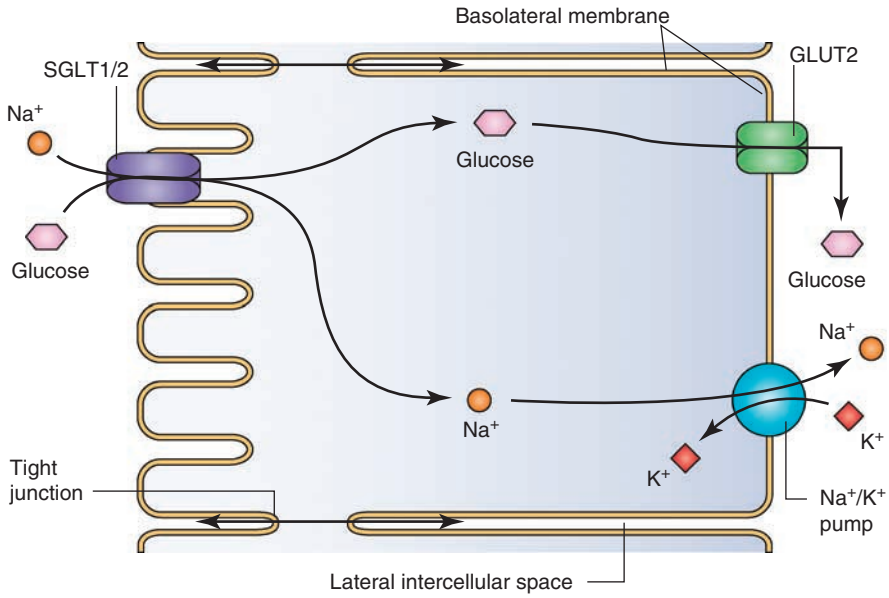

Figure 1 | Glucose reabsorption from the glomerular filtrate through a proximal tubule epithelial cell into the blood. Modified with permission from Wright *et al.*[3]

may contribute to the development of diabetic renal tubular and glomerular disease.[10]

CURRENT THERAPIES FOR HYPERGLYCEMIA IN TYPE II DIABETES AND UNMET NEEDS

Current non-insulin-based therapies for type II diabetes mellitus (DM2) include sulfonylurea-related secretagogues, metformin, the α-glucosidase inhibitors, incretin mimetics, DPP-4 inhibitors, and thiazolidinediones.[11–13] The α-glucosidase inhibitors inhibit carbohydrate digestion in the gut, whereas incretin mimetics slow gastric emptying, increase insulin secretion, and decrease glucagon secretion. The DPP-4 inhibitors also increase insulin secretion and decrease glucagon secretion, whereas the secretagogues simply stimulate secretion of insulin. Metformin and thiazolidinediones enhance insulin action in the liver and its periphery, respectively, and thereby increase glucose metabolism and suppress glucose production. Thiazolidinedione therapy also increases glucose intake and decreases free fatty acid output.[11–13]

Current therapies are associated with a number of important adverse events that can affect adherence to therapy or the health of the patient. Metformin is associated with gastrointestinal effects such as nausea and diarrhea, which occur in up to 50% of patients, and rarely, with lactic acidosis, and, importantly, is contraindicated in patients with moderate-to-severe renal disease.[14] The sulfonylureas and meglitinides are associated with hypoglycemia, weight gain, and hyperinsulinemia; and weight gain and edema can occur with the use of thiazolidinediones. α-Glucosidase inhibitors may cause malabsorption symptoms in patients,[14] whereas the incretin mimetics have gastrointestinal effects such as nausea, vomiting, and diarrhea.[15–18]

Given the progressive nature of DM2, most patients eventually require combinations of oral antidiabetic medications.[14] The United Kingdom Prospective Diabetes Study (UKPDS) found that after 3 years, only 50% of patients were adequately controlled on a single medication, and that after 9 years, only 25% were controlled on monotherapy.[19] The reasons for the difficulty in achieving glycemic goals are complex. Although the reluctance of medical professionals and patients to intensify treatment may contribute, it seems likely that the progression of the underlying disease process, together with dose-limiting side effects of treatment, contributes to the poor glycemic control that is common in DM2. The recent ACCORD study showed an increase in mortality when attempts at achieving normoglycemia were made with multiple combinations of these drugs.[20] Thus, better and safer therapy is needed to prevent the complications of diabetes.

SGLT INHIBITION: PRECLINICAL DATA

The classic inhibitor of sodium-dependent glucose transport is phlorizin, and it is well known that oral delivery produces glucosuria in both animals and humans, inhibits glucose absorption, and produces diarrhea.[21] This plant glucoside is a potent competitive inhibitor of SGLT1 and SGLT2, with submicromolar K_is, but is only a poor inhibitor of facilitated glucose transporters (GLUTs), $K_i > 0.5$ mM.[9] Phlorizin treatment of male diabetic Wistar rats was recently compared with insulin and a placebo.[10] Insulin injection resulted in a 4-h decreased plasma glucose, which remained low at 12 h. Phlorizin produced a similar significant decrease in plasma glucose ($P < 0.001$ for both phlorizin and insulin at 4 h as compared with placebo). However, insulin-treated rats experienced hyperinsulinemia, as expected, but insulin levels remained low in the phlorizin-treated rats. Insulin treatment resulted in rapid weight gain, and 4- and 6-day-treated rats were 16 and 20% heavier, respectively, than placebo-treated rats ($P < 0.05$).[10] In contrast, phlorizin-treated rats did not experience change in weight or insulin concentration. Insulin treatment lowered plasma glucose by 24% after 1 day of treatment ($P < 0.01$) and 60% after a 6-day regimen ($P < 0.001$), whereas phlorizin treatment resulted in a 56% decrease in plasma glucose after 1 day and a 55% decrease on days 4 and 6 ($P < 0.001$).[10]

Over 21 SGLT inhibitors are in the drug pipeline, and most exploit the chemical space of phlorizin. One of the first was the oral prodrug T-1095.[22] The prodrug did not inhibit intestinal glucose absorption, but its metabolite T-1095A effectively blocked renal glucose reabsorption. The usefulness of this SGLT inhibitor was explored in a study on diabetic and normoglycemic male mice.[23] It was proposed that the higher SGLT activity in diabetic mice than in non-diabetic mice would provide the rationale for the use of an SGLT inhibitor as DM2 therapy.[23] A single oral administration of T-1095 to diabetic mice resulted in a dose-dependent reduction in blood glucose levels and an increase in glucose excretion in the urine. Blood glucose levels of non-diabetic control mice were only slightly affected by the compound. Chronic administration of T-1095 resulted in decreases in both blood glucose and HbA_{1c} levels in diabetic mice, as well as 2.5-fold increases in pancreatic levels of insulin (Figure 2). In addition, progression of diabetic nephropathy was prevented, with suppression of both development of albuminuria and expansion of the glomerular mesangial area.[23] This study provided a proof of principle of targeting renal SGLTs in diabetics.

SELECTIVE SGLT2 INHIBITION

Selective SGLT2 inhibitors offer the potential to affect only the kidney, allowing excretion of glucose and creation of a negative energy balance without a potential gastrointestinal side effect profile. Two SGLT2 inhibitors currently under investigation are dapagliflozin, a C-aryl glucoside, and sergliflozin, an *O*-glucoside.[24,25]

Dapagliflozin has a 1200-fold selectivity for SGLT2 over SGLT1 as compared with phlorizin's 10-fold selectivity.[24] *In vitro* studies indicate that the inhibitory potencies against rat SGLT2 and human SGLT2 are comparable.[24] When administered to streptozotocin-induced diabetic rats, dapagliflozin resulted in an ~55% reduction in blood glucose as compared

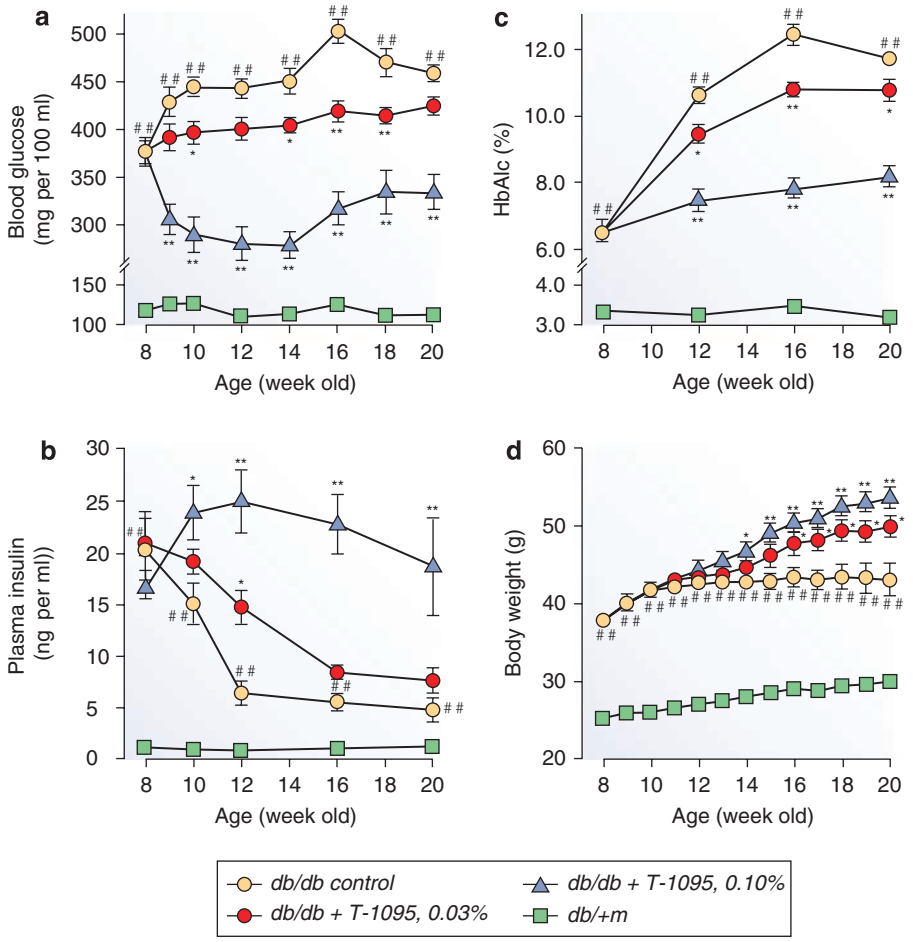

Figure 2 | Effects of chronic T-1095 treatment on blood glucose, HbA1C, plasma insulin, and body weight. Effects of chronic T-1095 treatment on blood glucose (**a**), plasma insulin (**b**), HbA1C (**c**), and body weight (**d**) in C57BL/KsJ-db/db (db/db) mice, a genetic animal model of obese type 2 diabetes. T-1095 (0.03 and 0.10% in diet) was given for 12 weeks. Blood glucose, HbA1C, plasma insulin levels, and body weight were monitored periodically. Symbols represent mean values and vertical lines show s.e. mean ($n = 8$). $^{\#\#}P < 0.01$ versus db/+m mice. $^{*}P < 0.05$, $^{**}P < 0.01$ versus control. Reprinted with permission from Arakawa *et al.*[23]

with controls at 5 h after a single dose.[24] Lower doses produced less glucose lowering in diabetic rats.

A recent study[26] provides additional evidence to support the potential of SGLT2 inhibitors to lower blood glucose. The investigators used cell-based assays in human SGLT1- and SGLT2-cloned cell lines to measure glucose analog uptake and assess dapagliflozin's ability to inhibit sodium-dependent and facilitative glucose transport activity. In addition, acute and multi-dose studies were conducted in normal and Zucker diabetic fatty rats to evaluate the effect of dapagliflozin on fed and fasting plasma glucose levels. Finally, a hyperinsulinemic euglycemic clamp study was performed to explore the compound's ability to improve glucose utilization after multi-dose treatment.

The *in vitro* study showed that dapagliflozin was approximately 30-fold more potent than phlorizin against human SGLT2 and approximately 4-fold less potent against human SGLT1. Dapagliflozin did not significantly inhibit facilitative glucose transport in human adipocytes.[26] The *in vivo* studies showed that dapagliflozin induced significant, dose-dependent glucosuria in both normal and Zucker diabetic fatty rats, enhanced glucose tolerance in normal rats, and decreased hyperglycemia in Zucker diabetic fatty rats after single oral doses ranging from 0.1 to 1.0 mg/kg.[26] Two-week treatment with once-daily dapagliflozin lowered fasting and fed glucose levels significantly at doses ranging from 0.1 to 1.0 mg/kg (Figure 3). No body weight changes compared with vehicle-treated rats were observed. Furthermore, use of the compound caused a significant increase in the glucose utilization rate, which was accompanied by a significant reduction in glucose production and enhanced liver insulin sensitivity.[26] Hypoglycemia was not observed in any of the groups during the course of the acute and chronic studies.[26]

Sergliflozin-A is a highly potent and selective inhibitor of SGLT2. In rats, mice, and dogs, oral administration of sergliflozin results in increased urinary glucose excretion in a dose-dependent manner. It also significantly lowers plasma glucose in a dose-dependent manner when administered to streptozotocin-induced diabetic rats without stimulating

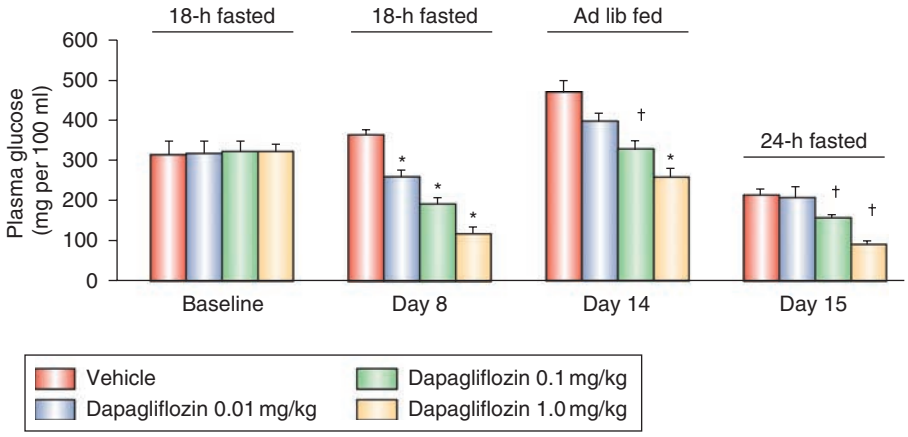

Figure 3 | Dapagliflozin lowers fasting and fed plasma glucose over 15 days of once-daily oral treatment in Zucker diabetic fatty rats. *$P<0.0001$; †$P<0.05$; each versus vehicle.[26] Copyright © 2008 American Diabetes Association. Reprinted with permission from the American Diabetes Association.

insulin secretion.[25] It does not significantly alter normal glucose levels in 6-h fasted rats, however, suggesting that the risk of hypoglycemia is low. Unlike phlorizin, which is hydrolyzed to phloretin, sergliflozin does not appear to have any effect on GLUT1 at pharmacological concentrations.[25] Nor does sergliflozin have an effect on urinary electrolyte excretion except for a temporary osmotic diuresis that occurs only at the highest dose.[25]

CONCLUSIONS

High plasma glucose levels appear to have a deleterious effect on the kidneys in those with diabetes. SGLT2 plays an important role in reabsorption of glucose in the proximal renal tubule. Animal studies suggest that selective inhibition of SGLT2 reduces plasma glucose levels without inducing insulin secretion, hypoglycemia, or weight gain. Two SGLT2 inhibitors, dapagliflozin and sergliflozin, are currently under investigation as possible therapies in DM2, with promising animal data. However, much remains to be explored with these compounds. The long-term effects of glucosuria need to be determined. In addition, these drugs are lipid-soluble, and therefore will cross the blood–brain barrier. The SGLTs are found within the hypothalamus, where they sense and respond to changes in glucose concentrations.[27] It is therefore critical that potential effects such as these are explored to determine the safety of these compounds.

It is clear, however, from the low rates of glucose control in the diabetic population that there is a need for an alternative approach to management of diabetes. Targeting the SGLT2 transporter in the kidney seems to be a very promising approach for the treatment of DM2.

DISCLOSURE

George Bakris
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