Role of Sodium-Glucose Cotransporter 2 (SGLT 2) Inhibitors in the Treatment of Type 2 Diabetes

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Hyperglycemia plays an important role in the pathogenesis of type 2 diabetes mellitus, *i.e.*, glucotoxicity, and it also is the major risk factor for microvascular complications. Thus, effective glycemic control will not only reduce the incidence of microvascular complications but also correct some of the metabolic abnormalities that contribute to the progression of the disease. Achieving durable tight glycemic control is challenging because of progressive β-cell failure and is hampered by increased frequency of side effects, *e.g.*, hypoglycemia and weight gain. Most recently, inhibitors of the renal sodium-glucose cotransporter have been developed to produce glucosuria and reduce the plasma glucose concentration. These oral antidiabetic agents have the potential to improve glycemic control while avoiding hypoglycemia, to correct the glucotoxicity, and to promote weight loss. In this review, we will summarize the available data concerning the mechanism of action, efficacy, and safety of this novel antidiabetic therapeutic approach. **(*Endocrine Reviews* 32: 515–531, 2011)**

I. Introduction

Type 2 diabetes mellitus (T2DM) is the most common metabolic disease and is associated with considerable morbidity and mortality (1–7). Many epidemiological studies have demonstrated that hyperglycemia is the major risk factor for microvascular complications (8, 9). Hyperglycemia not only represents the biochemical marker by which the diagnosis of diabetes is made but also plays a pivotal role in the pathogenesis of the two core defects characteristic of T2DM: insulin resistance and β-cell failure, *i.e.*, glucotoxicity (10–12). Thus, improved glycemic control in diabetic subjects not only reduces the risk of microvascular complications but also ameliorates the metabolic abnormalities that contribute to the progressive course of the disease. Therefore, tight glycemic control has become the cornerstone of management in subjects with T2DM, and all professional organizations recommend that the glycosylated hemoglobin (HbA_{1c}) should be maintained at 6.5–7% or less (13–16).

Progressive β-cell failure, weight gain, and hypoglycemia represent major obstacles for the achievement of good glycemic control (HbA_{1c}, 6.5%–7% or less) in patients with T2DM (11). Moreover, T2DM individuals are characterized by multiple metabolic defects involving multiple

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Abbreviations: ASO, Antisense oligonucleotides; FFA, free fatty acid; GFR, glomerular filtration rate; GLUT, glucose transporter; HbA_{1c}, glycosylated hemoglobin; HGP, hepatic glucose production; OGTT, oral glucose tolerance test; SAAT1, sodium-amino acid transporter; SGLT, sodium-glucose cotransporter; T2DM, type 2 diabetes mellitus; Tm, maximum glucose transport capacity.

pathways in numerous organs, a pathogenesis which has been referred to as the "ominous octet" (11). Therefore, the development of novel medications, which effectively lower the plasma glucose level, produce durability of glycemic control, and are not associated with hypoglycemia and weight gain, is needed for the management of T2DM patients. Most recently, inhibitors of the renal sodium-glucose cotransporter have been developed to produce glucosuria and reduce the plasma glucose concentration. In this review, we will summarize the available data concerning the mechanism of action, efficacy, and safety of this novel antidiabetic therapeutic approach.

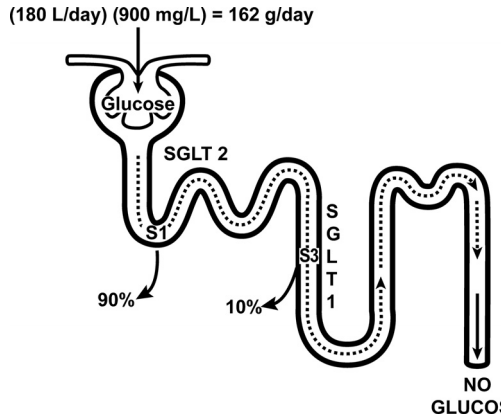

Fig. 1. Renal tubular regulation of glucose reabsorption. See *Section III* for a detailed description.

II. Glucose Transport across Cell Membranes

Glucose is an essential fuel source for cellular metabolism. The glucose molecule is highly polar and does not cross the lipid bilayer that comprises the plasma membrane of all living cells. Because of this, membrane proteins that facilitate glucose transport from the extracellular to the intracellular space are pivotal for glucose movement across cell membranes. Two distinct classes of glucose transporters exist in the human body (17, 18): 1) facilitative glucose transporters (GLUT), a family of proteins that passively facilitate glucose movement from the extracellular to the intracellular space along its chemical gradient and, thus, do not consume energy (17, 18); and 2) sodium-glucose cotransporters (SGLT), a family of proteins that actively transport glucose across cell membranes against its concentration gradient, thereby requiring an energy source for their action. At least 14 different GLUT and seven SGLT have been identified. SGLT couple glucose with sodium transport into the cell (17, 18). Because sodium is transported along its electrochemical gradient, it provides the energy required for SGLT to transport glucose against its concentration gradient into the cell. SGLT mediate glucose transport across the intestinal lumen and across the epithelial cell in the proximal renal tubule (19).

Both GLUT and SGLT are large-membrane proteins. GLUT have 12 transmembrane domains, and more than 12 different types of GLUT have been described (18). Two different sodium-glucose cotransporters have been described, SGLT1 and SGLT2 (18, 19); both are large-membrane proteins (670 amino acids), and each has 14 transmembrane domains. The homology between SGLT1 and SGLT2 is approximately 58% (20, 21).

III. Filtration of glucose by the kidney

The kidney plays a pivotal role in the regulation of the plasma glucose concentration. Approximately 180 liters of plasma with a glucose concentration of approximately 90 mg/dl are filtered by the glomeruli every day. The filtered plasma, in addition to water, salts, and amino acids, contains approximately 162 g of glucose each day. In normal glucose-tolerant subjects virtually all of this glucose is completely reabsorbed in the proximal tubule. The net result is that no glucose is excreted in the urine. Glucose transport from the lumen across the apical membrane of the epithelial cell occurs against a concentration gradient and, therefore, requires an active transport process. The early convoluted segment (S1) of the proximal tubule reabsorbs approximately 90% of the filtered renal glucose. This is accomplished by the high-capacity, low-affinity SGLT2 transporter. The remaining 10% of the filtered glucose is reabsorbed by the high-affinity, low-capacity SGLT1 transporter in the distal straight segment (S3) of the proximal tubule (21, 22) (Fig. 1). Both SGLT1 and SGLT2 couple glucose transport to the sodium gradient, and the sodium electrochemical gradient generated by active sodium transport provides the energy required for glucose transport. After glucose has been transported into the renal proximal tubular cell by the SGLT2 transporter, the sugar exits the basolateral cell border via the GLUT2 transporter. The maximum glucose transport capacity (Tm) of the proximal tubule varies between individuals and, on average, has a value of approximately 375 mg/min (21). Because the filtered glucose load is less than 375 mg/min in nondiabetic subjects, all of the filtered glucose is reabsorbed and returned to the circulation (Fig. 1). The amount of filtered glucose is directly related to the plasma glucose concentration. If the filtered glucose load exceeds 375 mg/min, as may occur in T2DM subjects, the Tm is exceeded, and all glucose in excess of the Tm is excreted in the urine (Fig. 2). The plasma glucose concentration at which the filtered glucose load reaches 375 mg/min is called the threshold. When the threshold is exceeded, the glucose excretion rate increases linearly and parallels the filtered load. The reabsorption and excretion curves display a nonlinear transition as the Tm for glucose is approached (Fig. 2). This "rounding" of the curves is termed

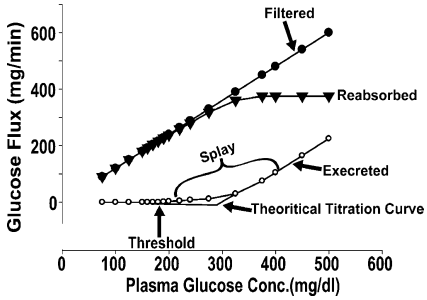

Fig. 2. Glucose reabsorption and excretion by the kidney. See *Section III* for a detailed discussion.

splay, and it has been explained by heterogeneity in the Tm for individual nephrons and/or glomerulotubular imbalance, *i.e.*, tubular reabsorption is not in balance with the glomerular filtration rate (GFR).

IV. Sodium-Glucose Cotransporters

Six different genes that encode for sodium-glucose cotransporters have been isolated in humans (19, 20, 23, 24).

Only the SGLT1 and SGLT2 have been well characterized in man, and their role in gut and kidney glucose transport, respectively, has been well defined (19).

The SGLT1 gene was first cloned from an intestinal rabbit cDNA library (20). The human SGLT1 gene is located at chromosome 22 q13.1 and spans 72 kb of genomic DNA (23). It is highly conserved throughout evolution, and more than 55 members of SGLT1 have been described in bacteria, yeast, invertebrates, and vertebrates. The human SGLT1 gene encodes a 670-amino acid protein, the SGLT1 transporter, which is found in the intestinal mucosa where it transports glucose and galactose from the intestinal lumen across the intestinal mucosa. SGLT1 is also expressed in the S3 segment of the renal proximal tubule (24). SGLT1 transports both glucose and galactose with similar affinity for both molecules. It has a glucose/galactose to sodium stoichiometry of 2:1 (Table 1) and possesses a high affinity for both glucose and galactose (Km = 0.2 mM) but a low transport capacity (Tmax = 2 nmol/mg protein · min) (25).

The SGLT2 gene is located at chromosome 13 p11.2 (26), and it is expressed primarily in kidney cortex (19). The SGLT2 transporter also is expressed at low levels in the brain and liver (21). It is the principal glucose transporter in the renal proximal tubule, and it is highly selective for glucose over galactose. It has a low affinity for glucose (Km = 2 mM) with high transport capacity (Tmax = 10 nmol/mg protein · min), and it transports one glucose molecule for every sodium ion (27).

A third SGLT gene has been isolated from a pig renal cell line with a rabbit SGLT1 probe. It has 70% homology

TABLE 1. Anatomical location and biochemical characteristics of the SGLT1 and SGLT2 transporters

	SGLT1	SGLT2
Renal location	S3 segment of proximal tubule	S1 segment of proximal tubule
Extrarenal location	Gut, heart, RBC	Brain, liver
Sugar selectivity	Glucose = galactose	Glucose >> galactose
Na/glucose stoichiometry	1:2	1:1
Glucose affinity	High (0.4 mM)	Low (2 mM)
Glucose transport capacity	Low (2 nmol/mg · min)	High (10 nmol/mg · min)
Clinical syndrome resulting from mutation	Diarrhea	Glucosuria

See *Section IV* for more detailed discussion. RBC, Red blood cells.

to SGLT1 and, in expression experiments in oocytes, facilitates amino acid transport into the oocyte. Thus, it initially was designated as a sodium-amino acid transporter (SAAT1) (28). Subsequent studies have demonstrated that the protein encoded by the SAAT1 gene also facilitates sodium-glucose transport with high selectivity and low affinity to glucose. Thus, it has been referred to as the pig SGLT2. A human analog of the pig SGLT2 gene has been identified and located at chromosome 22 in close proximity to the SGLT1 gene (within 0.15 ml), and it has a similar intron-exon organization to SGLT1 (29). The homology between SAAT1 and SGLT1 and the close proximity in the location of the two genes suggest an ancient gene duplication. Recent studies have demonstrated that the human SGLT3 gene encodes a 659-amino acid protein that is expressed in skeletal and smooth muscle and in neurons, including the enteric neuron (30). Unlike SGLT1 and SGLT2, binding of glucose to SGLT3 activates sodium efflux from the cell, leading to membrane depolarization without glucose transport (30). SGLT3 activation with glucose in the enterochromaffin cells leads to cell depolarization and stimulation of serotonin secretion that modulates vagal activity and gastrointestinal motility (31). It has been suggested that SGLT3 functions as a glucose sensor rather than a glucose transporter.

Two additional sodium-glucose transporters, SGLT4 and SGLT5, have been identified (24). SGLT4 is a low-affinity transporter for glucose and mannose, and the gene is expressed in a variety of tissues including the pancreas (24). The SGLT5 gene is exclusively expressed in the renal cortex. However, the protein encoded by SGLT5 gene has not been identified, nor has its function been elucidated (24).

V. Familial Renal Glucosuria

Familial renal glucosuria is characterized by urinary glucose excretion in the presence of a normal blood glucose

TABLE 2. EC_{50} (in nM) for human SGLT1 and SGLT2 inhibition in Chinese hamster ovary cells stably transfected with human SGLT genes

	SGLT1	SGLT2	Selectivity for SGLT 2 *vs.* SGLT1
Phlorizin	35.6	330	10
T-1095	6.6	211	30
Sergliflozin	9.2	>8000	>90
Dapagliflozin	1.1	1390	1200

See *Section VII* for a more detailed discussion.

concentration and the absence of other signs of general renal tubular dysfunction (32). Genetic analysis of families with renal glucosuria has demonstrated that this disorder is caused by mutations in the gene encoding for the SGLT2 transporter, and at least 21 different mutations in the SGLT2 gene have been described (33). The majority of reported SGLT2 mutations are nonsense or frameshift mutations that result in disruption of transmembrane domains 10–13, which are essential for sugar binding and sugar transport by the SGLT2 protein (Table 2). Most of these mutations are inherited in an autosomal recessive mode, and the index subject is either homozygous or compound heterozygous. The severity of glucosuria varies markedly among affected individuals, ranging from 20 to 200 g of glucose per 24 h. Of note, affected individuals are asymptomatic and have no history of growth retardation, polyuria, polydipsia, renal disease, or increased urinary tract infection (33).

Recently, an SGLT2 knockout mouse has been created (34). Mice lacking the SGLT2 transporter had glucosuria, polyurea, and increased food and fluid intake. However, compared with wild-type animals, the SGLT2 knockout mice had comparable body weight, plasma glucose concentration, GFR, and urinary excretion of electrolytes and amino acids. Free-flow micropuncture studies demonstrated a marked decrease in glucose reabsorption in the proximal tubule (6% of the glucose load *vs.* 78% in the wild type) (34). These mutations and the SGLT2 knockout mice collectively emphasize the central role of the SGLT2 transporter in renal glucose reabsorption. They also provide proof of concept that pharmacological inhibition of SGLT2 is a safe and potentially effective strategy for reducing the plasma glucose concentration in diabetic subjects. However, it is important to note that adaptive mechanisms may have developed in prenatal life to compensate for the lack of SGLT2 activity, and these mechanisms may not exist during pharmacological inhibition of SGLT2.

VI. Hyperglycemia and Renal Glucose Reabsorption

In T1DM and T2DM individuals, hyperglycemia results in an increased filtered glucose load. This leads to increased glucose reabsorption via proximal renal tubular cells, and as long as the Tm for glucose is not exceeded, no glucose appears in the urine. From a purely theoretical standpoint, hyperglycemia, by increasing the interstitial glucose concentration, would be expected to attenuate the glucose concentration gradient across the basolateral membrane of the proximal renal epithelial cell and lead to impaired glucose efflux. However, studies in experimental animal models of diabetes (35–37) consistently have reported an increased rate of glucose reabsorption in the proximal tubule in hyperglycemic diabetic rats during uncontrolled diabetes.

The molecular mechanism responsible for increased renal glucose reabsorption during hyperglycemia involves an increase in the expression of glucose transporter genes in the proximal tubule. Increased SGLT2 gene expression has been reported in renal proximal tubular cells in experimental animals (38) and in humans (39). Renal tubular cells isolated from the urine of patients with T2DM have an increase in the expression of SGLT2 mRNA and protein (39) and demonstrate an increase in glucose transport (Fig. 3). Several studies also have reported an increase in GLUT2 gene expression in the kidney in models of spontaneous diabetes, *i.e.*, the Zucker diabetic rat (40) and in pharmacologically induced (streptozotocin) diabetes (35, 36). Furthermore, in experimental diabetic animal models, correction of the hyperglycemia with insulin or phlorizin reversed the increase in SGLT2 gene expression caused by hyperglycemia (38). Because both insulin and phlorizin (which have very different effects on the plasma insulin and urinary glucose concentrations) prevent the increase in SGLT2 gene ex-

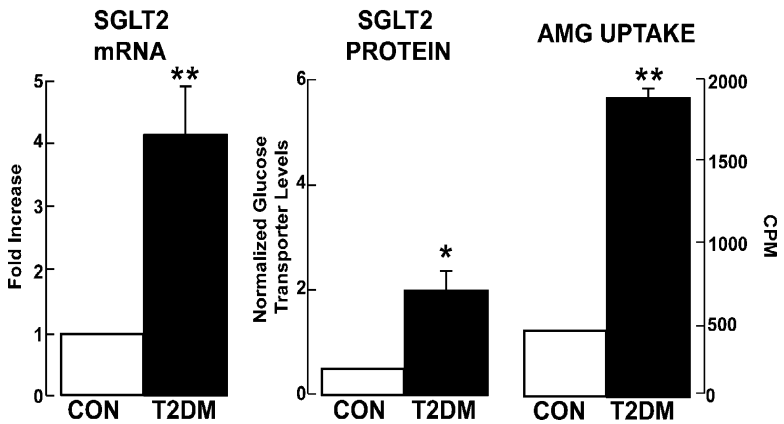

Fig. 3. SGLT2 mRNA and protein expression in cultured renal proximal tubular epithelial cells from T2DM and healthy control subjects (39). *, $P < 0.05$; **, $P < 0.01$.

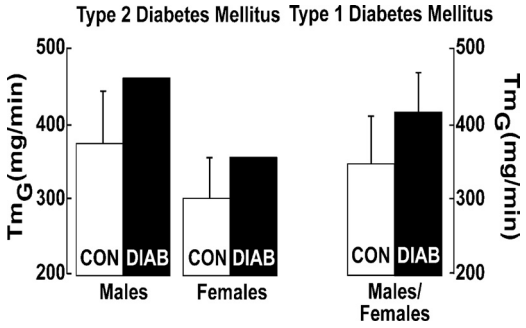

Fig. 4. Effect of hyperglycemia on the renal Tm for glucose in T2DM subjects (*left*) (from Ref. 40) and in T1DM subject (*right*) (from Ref. 41). CON, Control; DIAB, T2DM.

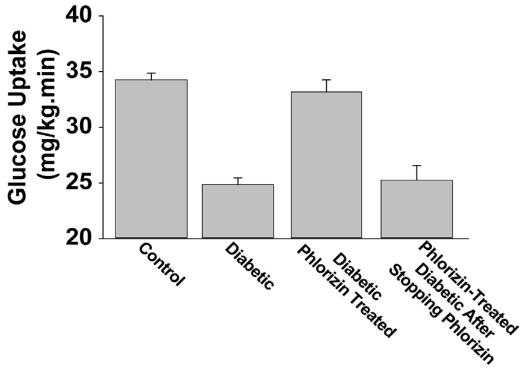

Fig. 5. Effect of phlorizin therapy on insulin sensitivity in partially pancreatectomized diabetic rats (50).

pression caused by hyperglycemia, it is likely that the elevated plasma glucose concentration provides the stimulus that ultimately leads to increased SGLT2 and GLUT2 mRNA/protein expression by the renal proximal tubular cells (39). In both T2DM (41) and T1DM (42) patients, the renal Tm for glucose also has been shown to be increased (Fig. 4). The study by Farber *et al.* (41) is of particular interest because correction of the hyperglycemia resulted in a decrease in Tm for glucose and the appearance of glucosuria. Thus, almost 50 yr ago investigators already had demonstrated an increase in the renal Tm for glucose in response to chronic hyperglycemia.

Normal glucose-tolerant subjects have a Tm for glucose that is well above the filtered glucose load. This has major survival benefits because it allows the kidneys to conserve this critical energy source for the brain, which (with the exception of prolonged fasting) only can metabolize glucose to generate energy for neuronal function. However, in the diabetic patient this adaptive mechanism now becomes maladaptive. In the presence of hyperglycemia, it would be desirable for the kidney to excrete the excess filtered glucose load to restore normoglycemia. In contrast (see *Section VI*), the diabetic kidney has an increased Tm for glucose, thereby minimizing glucosuria and exacerbating the hyperglycemia. When viewed in these terms, it is evident that the kidney contributes to the development and maintenance of hyperglycemia in individuals with diabetes. Based upon these pathophysiological considerations, it follows that development of inhibitors of the renal SGLT2 transporter provides a rational and novel approach to the treatment of diabetic patients. It is important to avoid inhibition of the SGLT1 transporter (which is present in both the gut and kidney), because this would lead to glucose malabsorption and diarrhea. Recent evidence also suggests that the SGLT1 transporter in cells of the proximal small intestine may be responsible for generating the signal leading to the release of incretin hormones in response to nutrient ingestion (43).

VII. Pharmacological Inhibitors of Renal Glucose Uptake

A. Phlorizin

Phlorizin is comprised of a glucose moiety and two aromatic rings joined by an alkyl spacer, and was first isolated in 1835 by a French chemist from the bark of apple trees (44). In 1886, Von Mering demonstrated that ingestion of high doses of phlorizin (>1 g) produced glucosuria in man (45). Subsequent studies in the 1950s focused on the cellular mechanism of phlorizin action and demonstrated that lower concentrations of phlorizin blocked facilitated glucose transport in erythrocytes, kidney, and small intestine (46). Further research demonstrated that the glucosuric action of phlorizin resulted from inhibition of active glucose transport in the apical membrane of the renal proximal tubule (47). Phlorizin competitively inhibits both SGLT1 and SGLT2 in the proximal tubule with a higher affinity (10-fold) for the SGLT2 *vs.* SGLT1 transporter and, when given to normal subjects, produces glucosuria that resembles familial renal glucosuria (48). In nondiabetic animals, iv administration of phlorizin produces glucosuria with minor or no change in the plasma glucose concentration (49). However, in diabetic animals, phlorizin treatment normalized the plasma glucose concentration (50–52), suggesting that pharmacological inhibition of SGLT2 activity in the kidney is an effective strategy for glycemic control in diabetic subjects. Despite the efficacy of phlorizin in inhibiting SGLT activity and normalizing the plasma glucose concentration in diabetic animals, several properties negate its clinical usefulness in subjects with diabetes (53): Firstly, after oral administration, the majority of phlorizin is converted to phloretin in the gut by the enzyme disaccharidase (54); therefore, the bioavailability of oral phlorizin is very low (~15%). Secondly, phloretin, a metabolite of phlorizin, is a potent inhibitor of both GLUT2- and GLUT1-mediated glucose absorption across the brush-border membrane of the gut. At high plasma concentrations, it

can inhibit insulin secretion and insulin-stimulated glucose transport. However, with the low doses employed by Rossetti *et al.* (12, 50–52), both insulin secretion and insulin sensitivity were returned to normal in partially pancreatectomized rats (52). Lastly, phlorizin has low selectivity for SGLT2 compared with SGLT1 (Table 2). Thus, gastrointestinal side effects are frequent after phlorizin administration. Because of these limitations of phlorizin, other compounds with greater bioavailability after oral administration and higher selectivity for SGLT2 compared with SGLT1 have been developed.

B. T-1095

T-1095 is a phlorizin derivative with higher bioavailability after oral administration (55). It was developed by Tanabe Seiyaku Co. by the addition of a methyl-carbonate group to phlorizin to prevent its degradation in the gut by glucosidase. T-1095 is a prodrug and, after its oral administration, is metabolized in the liver to T-1095A, the active form of the compound. T-1095A acts on the proximal tubule to inhibit SGLT2 and produce glucosuria (55). The prodrug, T-1095, also inhibits SGLT1 and, after its oral administration, has the potential to inhibit the intestinal SGLT1 transporter. However, the Ki for SGLT1 inhibition by T-1095 is 6–120 times higher than the Ki for SGLT2 inhibition by T-1095A, depending upon the animal species studies (55). Therefore, the major hypoglycemic action of T-1095 results from inhibition of SGLT2 by its active metabolite.

In vitro studies have demonstrated that T-1095A has a 30-fold specificity to inhibit human SGLT2 compared with SGLT1 when it is expressed in *Xenopus* oocytes (Table 2). In brush-border membrane vesicles prepared from the kidney of mouse, rat, and dog, T-1095A inhibits glucose-dependent glucose transport with a Ki ranging from 0.66 to 1.5 μM (56).

In normal and diabetic animals, oral administration of T-1095 produces a dose-dependent increase in urinary glucose excretion (55). The maximal glucosuric effect of the drug is achieved with a dose of 300 mg/kg, and this dose causes marked glucosuria in normal and diabetic rats (1 g/100 g body weight per 24 h) (55). Kinetic analysis of the inhibition of SGLT2 with T-1095 demonstrated that T-1095 causes glucosuria by decreasing the Tm for glucose reabsorption in the proximal tubule (57). However, because of the nonselective nature of the drug and concerns over its safety, T-1095 was discontinued after phase II clinical trials.

C. Sergliflozin

Sergliflozin was developed by Kissei Pharmaceutical Co. in Japan and currently is being developed for the treatment of T2DM by GlaxoSmithKline. It is a more potent inhibitor for renal glucose reabsorption than T-1095 (58). Importantly, sergliflozin also has a higher selectivity for human SGLT2 (59). In cells expressing human SGLT2 and SGLT1, sergliflozin inhibits both transporters in a dose-dependent manner, but it has 296-fold selectivity for SGLT2 *vs.* SGLT1 (Ki = 2.39 and 708 nM, respectively) (Table 2). Similar to T-1095, sergliflozin is converted *in vivo* to an active metabolite, sergliflozin A (58). However, unlike phloretin (a phlorizin metabolite), neither sergliflozin A nor T-1095A exerts any significant inhibitory effect on GLUT2. Studies in experimental animals have demonstrated that oral administration of sergliflozin induces a dose-dependent glucosuria in rats, mice, and dogs. An oral dose of 30 mg/kg caused significant glucosuria in dogs (1 g/kg per 24 h) and rats (2 g/kg per 24 h) (58). Studies in rats have demonstrated that sergliflozin reduces the Tm for glucose by more than 60% without apparent effect on the glucose splay (59). In a single-dose pharmacodynamic/pharmacokinetic study in man, sergliflozin caused a dose-related (5, 15, 50, 100, 200, and 500 mg) glucosuria under fasting conditions and after glucose loading (60). However, the 24-h glucose excretion at the two highest doses was only 13 and 19 g, respectively, representing only 18 and 27%, respectively, of the filtered glucose load (60). However, it is possible that further dose escalations would have caused a greater increase in 24-h glucose excretion because it is clear that the maximally effective dose of sergliflozin was not achieved. Like T-1095, sergliflozin has been discontinued after phase II clinical trials.

D. Dapagliflozin

Dapagliflozin is being developed by Bristol Myers Squibb/Astra Zeneca. It has greater efficacy in inhibiting renal glucose reabsorption and possesses greater selectivity for SGLT2 *vs.* SGLT1 compared with both T-1095 and sergliflozin. Dapagliflozin has 84% bioavailability in rats and a pharmacological half-life of 4.6 h (61). Dapagliflozin circulates bound to albumin and, at a plasma concentration of 10 μM, the free fraction is 3 and 4% in rat and man, respectively (61). In CHO cells expressing SGLT1 and SGLT2, dapagliflozin has an approximately 1200-fold selectivity for SGLT2 *vs.* SGLT1 (Ki = 1.1 and 1390 nM for SGLT2 and SGLT1, respectively) (61). Of note, the selectivity of dapagliflozin for rat SGLT2 *vs.* SGLT1 is only approximately 200-fold. In *in vitro* assays, dapagliflozin has been shown to be 6- and 8-fold more potent in inhibiting the human SGLT2 transporter expressed in CHO cells compared with T-1095 and sergliflozin, respectively (Table 2).

In *in vivo* studies, administration of dapagliflozin to normal and diabetic rats causes a dose-dependent glucos-

uria (62). When given as a single oral dose of 0.1, 1, and 10 mg/kg in normal rats, glucose excretion increased to 2.75, 5.5, and 9.5 g/kg body weight per 24 h (62). The effect of dapagliflozin on the kinetic properties of renal glucose transporter currently is unknown.

E. Other SGLT2 inhibitors

There are number of other SGLT2 inhibitors currently under development or in clinical trials. 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/d for 12 wk reduced the HbA_{1c} by 0.7–0.9% from baseline and 0.5–0.7% *vs.* placebo in association with weight loss of 1.3–2.3% (63). The 300 mg/d dose appeared to be slightly more effective than the lower doses. In a 16-d trial, canagliflozin was shown to improve β-cell function in T2DM patients using a model-based method to calculate insulin secretion (64). In a small study involving 29 T2DM subjects suboptimally controlled (HbA_{1c} = 8.4%) with insulin, addition of canagliflozin at 100 and 300 mg/d for 28 d reduced the HbA_{1c} by 0.7 and 0.9%, respectively (65). 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 (66). At the 100-mg dose, BI10773 increased urinary glucose secretion to 74 g over 24 h and reduced the plasma glucose excretion during an oral glucose tolerance test (OGTT). In a 12-wk double-blind study, 361 Japanese T2DM patients treated with ASP1941 at doses ranging from 12.5 to 100 mg/d experienced a 0.9% reduction in HbA_{1c} at the two highest doses (50 and 100 mg/d) (67). Body weight also was dose-dependently reduced by up to 2 kg in the 100 mg/d dose. In a phase IIA study, LX4211, which inhibits SGLT2 and to a lesser extent SGLT1, at doses of 150 and 300 mg/d reduced the HbA_{1c} by 1.2%, but the starting HbA_{1c} (8.2–8.5%) was higher than in most other studies, and the placebo decreased the HbA_{1c} by 0.5% (68). AVE2268 by Sanofi-Aventis has currently initiated human trials with AVE2268 (69). 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 during an OGTT (69). Roche Pharmaceuticals (RG7201, phase II) also has SGLT2 inhibitors in early stages of development. Remogliflozin, which was developed by Kissei Pharmaceuticals and GlaxoSmithKline, has been discontinued, apparently to make way for development of the SGLT1 inhibitor (KGA-3235).

F. SGLT antisense oligonucleotides

Down-regulating SGLT2 gene expression in the kidney with antisense oligonucleotides (ASO) is a novel and exciting approach that has been used to inhibit renal glucose reabsorption. Studies in rats, dogs, and monkeys have demonstrated that ASO decrease renal SGLT2 mRNA expression by approximately 80% with no significant change in SGLT1 expression, and this is accompanied by pronounced glucosuria (70). Furthermore, a once-weekly injection of ASO for 4–5 wk caused a substantial reduction in plasma glucose concentration and HbA_{1c} without any appreciable side effects. Because the ASO work by reducing the SGLT2 protein content, rather than inhibiting the SGLT2 transporter, they have the potential to cause greater glucosuria and decrease the plasma glucose concentration more efficiently compared with pharmacological inhibition of SGLT2 activity.

VIII. Inhibition of Renal Glucose Transport Corrects Hyperglycemia: Proof of Concept

Studies performed with phlorizin in 90% pancreatectomized diabetic rats have provided proof of concept for the efficacy of SGLT2 inhibition in the treatment of T2DM. In this insulinopenic T2DM model (50–52), chronic phlorizin administration induced glucosuria and normalized both the fasting and fed plasma glucose levels with complete reversal of the insulin resistance (Fig. 5). When phlorizin was withdrawn from phlorizin-treated animals, hyperglycemia and insulin resistance returned. Chronic phlorizin treatment also corrected the defects in both first and second phase insulin secretion in this diabetic rodent model (50) (Fig. 6). Because phlorizin is poorly absorbed from the gastrointestinal tract and inhibits both the SGLT2 and SGLT1 transporters, it has not been developed commercially for the treatment of T2DM.

IX. Metabolic Effects of SGLT2 Inhibitors

A. Effect of SGLT2 inhibition on glucose metabolism in normal animals

Pharmacological inhibition of the SGLT2 transporter in normal animals results in significant glucosuria with

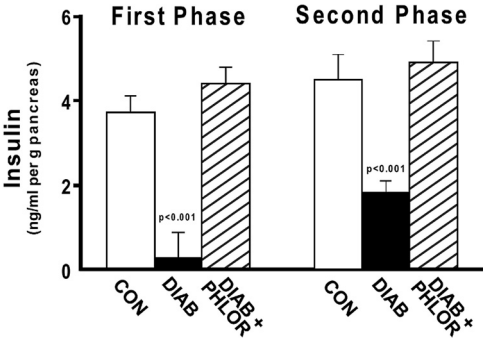

Fig. 6. Effect of phlorizin therapy on insulin secretion by the remnant pancreas in partially pancreatectomized diabetic rats (51).

only minimal or no change in the fasting plasma glucose concentration. Single-dose administration of all three inhibitors, T-1095 (56), sergliflozin (58), and dapagliflozin (62), causes only a small and transient (up to 6 h) decrease in plasma glucose concentration when administered to fasted normal rats; 24 h after drug administration, there was no significant change in the fasting plasma glucose concentration. Chronic treatment (>6 wk) of normal rats with SGLT2 inhibitors has no significant effect on the plasma glucose concentration despite marked dose-dependent glucosuria (58). The lack of significant effect of SGLT2 inhibition on the fasting plasma glucose concentration in normal animals, despite significant glucosuria, indicates that inhibition of renal glucose reabsorption activates counterregulatory mechanisms that increase endogenous (hepatic) glucose production (HGP) to precisely compensate for the increased urinary glucose loss. Although the effect of SGLT2 inhibitors on HGP has not been examined in normal animals, older studies with phlorizin in normal dogs reported that the glucosuria was associated with a marked increase in HGP with no significant change in arterial plasma glucose concentration (49). Of note, recycling of the dog's urine to the inferior vena cava blocked the increase in HGP after phlorizin infusion (49). The investigators failed to detect any significant change in plasma glucose concentration in the portal vein after phlorizin infusion and concluded that a decrease in the portal plasma glucose concentration could not be the trigger to increase HGP (50). However, the portal plasma flow is very high (1200 ml/min), and small changes in the portal glucose concentration would be difficult to detect. When phlorizin was infused and portal vein insulin, glucose, and glucagon concentrations were maintained with the pancreatic clamp technique, HGP remained unchanged (49). These results suggest that changes in pancreatic hormone secretion, *e.g.*, decreased insulin secretion and/or increased glucagon secretion, play an important role in the increase in HGP caused by phlorizin in normal animals. Consistent with this hypothesis, the fasting plasma insulin concentration decreased significantly after the administration of the SGLT2 inhibitor, T-1095, in normal animals (plasma glucagon concentration was not measured in this study) (56).

In contrast to the negligible effect of SGLT2 inhibitors on the fasting plasma glucose concentration in normal rats, a single dose of an SGLT2 inhibitor given 30 min before an oral glucose load reduced by 50% the area under the plasma glucose concentration curve. This effect on postprandial plasma glucose concentration has been observed with all three SGLT2 inhibitors (56, 58, 62). However, during chronic SGLT2 inhibitor treatment, there was no significant change in the area under the plasma glucose curve after glucose ingestion (56, 58, 62).

B. Effect of SGLT2 inhibition on glucose metabolism in diabetic animals

In diabetic animals, inhibition of the SGLT2 transporter has a very different effect on glucose metabolism compared with nondiabetic animals. When administered to streptozotocin-induced diabetic rats, a single oral dose (100 mg/kg) of T-1095 reduced the fasting plasma glucose concentration from approximately 18 mM to less than 5.5 mM (56). Similarly, a single oral dose of dapagliflozin reduced the fasting plasma glucose concentration in Zucker diabetic rats in a dose-dependent fashion, and the maximally effective dose (1 mg/kg) decreased the plasma glucose concentration from approximately 20 to approximately 5.5 mM (62). When given orally 30 min before glucose ingestion, both dapagliflozin and T-1095 markedly decreased the plasma glucose excursion (56, 57). Similarly, chronic treatment with SGLT2 inhibitors in diabetic animals, unlike normal animals, reduced both the fasting and fed plasma glucose concentrations. Twelve weeks of treatment with T-1095 in streptozotocin-induced diabetic rats (55) and 30 wk of treatment in the Goto-Kakizaki diabetic rat (71) caused a significant reduction in both fasting and postprandial plasma glucose concentrations, accompanied by reduction in HbA_{1c} from 12.6 to 7.2%. However, unlike normal animals, the decrease in plasma glucose concentration was accompanied by a marked decrease in the elevated rate of basal HGP (56). The decrease in plasma glucose concentration was accompanied by an increase in fasting plasma insulin concentration, which may have contributed to the decrease in HGP (56). Furthermore, plasma free fatty acid (FFA), which also was elevated in the diabetic rats, was normalized with T-1095 treatment (56). Because elevated plasma FFA levels stimulate hepatic gluconeogenesis and cause hepatic insulin resistance (72), normalization of the fasting plasma FFA concentration also could have contributed to the decline in basal HGP. Chronic hyperglycemia augments HGP by stimulating glucose-6-phosphatase, the rate-limiting enzyme for glucose exit from the hepatocyte, *i.e.*, glucotoxicity (73). Correction of the hyperglycemia with phlorizin down-regulates glucose-6-phosphatase, leading to a decline in HGP (52). Chronic hyperglycemia in T2DM subjects augments glucose uptake in muscle by mass action effect (74). Because both glucose oxidation and glycogen synthesis are markedly impaired in diabetic muscle (10), the glucose that is taken up in increased amounts during the basal postabsorptive state is released as lactate, which is returned to the liver where it stimulates hepatic gluconeogenesis, *i.e.*, acceleration of the Cori cycle. Correction

of the hyperglycemia, by reducing lactate production in muscle and adipocytes, would make less lactate available to the liver for gluconeogenesis.

C. Effect of SGLT2 inhibitors on insulin resistance

Patients with T2DM are characterized by two major core defects: 1) insulin resistance in skeletal muscle and liver; and 2) progressive β-cell failure (11). Hyperglycemia *per se* aggravates both the insulin resistance and β-cell dysfunction, and these deleterious effects of hyperglycemia have been referred to collectively as glucotoxicity (10).

The partially (90%) pancreatectomized diabetic rat provides an animal model for insulin-deficient T2DM. In this model, chronic hyperglycemia results in the development of severe insulin resistance in skeletal muscle and liver (50, 52). Phlorizin treatment for 4–5 wk normalized the fasting and postmeal plasma glucose concentrations in association with normalization of insulin-stimulated glucose disposal, measured with the euglycemic insulin clamp (Fig. 5). When phlorizin was withdrawn, both the hyperglycemia and severe insulin resistance returned. The improvement in insulin action was associated with a marked increase in 3-0-methylglucose transport in adipocytes without change in GLUT4 mRNA or protein (49). These results suggest that chronic hyperglycemia inhibits GLUT4 translocation and/or intrinsic activity.

Similar results have been reported in streptozotocin-induced diabetic rats treated with T-1095 for 12 wk. Whole body insulin-stimulated glucose disposal, measured *in vivo* with the insulin clamp, increased (56) in association with enhanced insulin-stimulated glucose uptake in skeletal muscle *in vitro* (75). Correction of the hyperglycemia in streptozotocin-treated animals with T-1095 decreased HGP to near normal values, indicating improved hepatic insulin resistance (56). Because neither phlorizin (52) nor T-1095 (56) has any effect on insulin-stimulated glucose disposal in nondiabetic rats, normalization of insulin sensitivity in the diabetic rat must be related to correction of the hyperglycemia, *i.e.*, amelioration of glucotoxicity. In Zucker diabetic rats (62), dapagliflozin also has been shown to augment insulin-stimulated glucose disposal in liver and to augment the suppression of HGP by insulin (62). Interestingly, in this 2-wk study dapagliflozin did not enhance insulin-stimulated glucose uptake in skeletal muscle or white adipocytes. Dapagliflozin does not inhibit facilitative glucose transport in human adipocytes (62).

In streptozotocin-treated rats, normalization of insulin-stimulated glucose disposal and suppression of HGP with T-1095 were associated with an improvement in insulin signaling in skeletal muscle and liver (76). In skeletal muscle, GLUT4 content increased, and the defect in insulin-induced GLUT4 translocation to the plasma membrane was corrected (56). In the liver, T-1095 treatment increased insulin receptor, insulin receptor substrate-1 and -2 tyrosine phosphorylation, and insulin-stimulated phosphatidylinositol-3 kinase activity (76). Taken collectively, the results described above emphasize the deleterious effect of hyperglycemia on insulin resistance in liver and muscle and demonstrate that, although the primary mechanism of action of the SGLT2 inhibitors is to inhibit glucose reabsorption by the kidney, by correcting the hyperglycemia they also ameliorate the muscle and hepatic insulin resistance.

D. SGLT2 inhibitors and β-cell function

Progressive β-cell failure, superimposed on underlying insulin resistance, is the primary abnormality responsible for worsening glycemic control in T2DM subjects (10, 11, 77). Chronically elevated plasma glucose levels inhibit insulin secretion *in vivo* in humans and animals and *in vitro* in cell culture systems (78–80). Conversely, normalization of plasma glucose levels with phlorizin in diabetic rats restores both first and second phase insulin secretion (51) (Fig. 6). Consistent with this glucotoxic effect of hyperglycemia on β-cell function, correction of hyperglycemia with intensive insulin therapy in human T2DM subjects improves insulin secretion (81). The precise level of hyperglycemia required to inhibit β-cell function in man is not known. However, in normal rats, a small (16 mg/dl) increment in mean daylong plasma glucose concentration reduces both first and second phase insulin secretion by 50% (78).

The plasma insulin response to treatment with an SGLT2 inhibitor depends on the glucose tolerance status of the animal. Chronic (12 wk) treatment with T-1095 in normal animals caused a small nonsignificant decrease in fasting plasma insulin concentration and insulin response during the OGTT (82). Conversely, in diabetic animals, T-1095 significantly increased both the fasting plasma insulin concentration and the plasma insulin response during the OGTT (56). Increased insulin response in the presence of a marked decrease in the plasma glucose concentration during the OGTT documents a robust improvement in β-cell function after restoration of normoglycemia with T1095. In Goto-Kakizaki diabetic rats, treatment (8 wk) with T-1095 enhanced first phase insulin secretion by approximately 30%, measured with the perfused pancreas technique (82). All of these studies are quite consistent and underscore the important role of glucotoxicity in the development of β-cell failure in T2DM. Furthermore, they demonstrate that correction of the elevated blood glucose

levels with a SGLT2 inhibitor has a favorable effect on β-cell function.

X. Human Studies

Clinical trials with dapagliflozin are the most advanced studies of the SGLT2 inhibitors, and several phase III trials have been completed. In a 14-d study, dapagliflozin (5, 25, and 100 mg/d) induced glucosuria (37, 62, and 80 g/24 h, respectively) and significantly reduced the fasting plasma glucose concentration (by 19, 29, and 39 mg/dl, respectively) and the area under the glucose curve during an OGTT in subjects with T2DM (83). Even at maximal doses, urinary glucose excretion accounted for only 40–50% of the filtered glucose load. In humans, the half-life time is approximately 17–18 h, making it suitable for once-daily administration (84). Dapagliflozin is rapidly absorbed after oral administration, achieving maximal plasma concentrations within 2 h. Moreover dapagliflozin is highly protein bound (97–98%), and renal excretion is low (2–4%). An inert glucuronoside conjugate (M15) of dapagliflozin is the major metabolite, and dapagliflozin does not inhibit or induce P450 enzymes. Because of its high plasma protein binding and low renal excretion, it is unlikely that the glucosuric action of dapagliflozin is mediated via the inhibition of the SGLT2 transporter by the concentration of dapagliflozin in the proximal tubular fluid. There is also no evidence to suggest that the glucosuric action of dapagliflozin is mediated by the concentration of dapagliflozin at the basolateral membrane. This raises the possibility that the M15 metabolite of dapagliflozin is responsible for the inhibition of glucose reabsorption in the proximal tubule. More prolonged treatment (12 wk) with dapagliflozin reduced the HbA_{1c} by approximately 0.7% without any apparent dose dependency (Table 3) in T2DM subjects with a baseline HbA_{1c} of 7.8–8.0% (85). The reduction in HbA_{1c} was of similar magnitude to that observed with metformin. Reductions in the fasting and postprandial plasma glucose concentrations accounted approximately equally for the decline in HbA_{1c} (Table 3). Dapagliflozin-treated diabetic subjects lost between 2.2 and 3.1 kg of body weight and also experienced a modest reduction in both systolic and diastolic blood pressure. The amount of glucosuria observed with dapagliflozin (50–60 g/d) is equivalent to a daily caloric loss of approximately 200–240 cal/d, which over the course of 12 wk could explain the 2–3 kg weight loss (86). In a large (n = 546), randomized, double-blind, placebo-controlled, 24-wk trial in metformin-treated T2DM patients, dapagliflozin in doses of 2.5, 5, and 10 mg/d reduced the HbA_{1c} by −0.67, −0.70, and −0.84%, respectively, compared with placebo (0.3%) (all $P < 0.01$) (87). Body weight was reduced by 2.26, 3.10, and 2.96 kg, respectively, compared with controls (−0.87 kg) (all $P < 0.01$) (87). In 485 T2DM patients controlled by diet and exercise, dapagliflozin in doses of 2.5, 5.0, and 10 mg/d reduced the HbA_{1c} by −0.58, −0.77, and −0.89% and body weight by −3.3, −2.8, and −3.2 kg after 24 wk (88). In the placebo-treated group, HbA_{1c} and body weight declined by −0.23% and −2.2 kg, respectively ($P < 0.001$ *vs.* dapagliflozin, 5 and 10 mg/d) (88). The incidence of urinary tract infection was: 4.0% (placebo), 4.6% (dapagliflozin = 2.5 mg/d), 12.5% (dapagliflozin = 5 mg/d), and 5.7% (dapagliflozin = 10 mg/d). The incidence of genital infections was: 1.3% (placebo), 7.7% (dapagliflozin = 2.5 mg/d), 7.8% (dapagliflozin = 5 mg/d), and 12.9% (dapagliflozin = 10 mg/d) (85). Rates of hypoglycemia and hypotension were similar in placebo and all dapagliflozin arms. There were no clinically relevant changes from baseline in serum creatinine or electrolytes. In a subgroup of 74 diabetic patients with HbA_{1c} = 10.1–12.0%, 24 wk of dapagliflozin treatment reduced the HbA_{1c} by 2.88% (5 mg/d) and 2.66% (10 mg/d) (85).

In an abstract presented at the 2010 European Association for the Study of Diseases meeting in Stockholm, Nauck *et al.* (89) compared the efficacy of dapagliflozin (n = 400) and glipizide (n = 401) in metformin-treated T2DM patients (age, 58 yr; body mass index, 31.4 kg/m^2; diabetes duration, 6.4 yr) with a starting HbA_{1c} of 7.7%. After 52 wk, the decrement in the HbA_{1c} was identical (−0.52%) in both treatment groups (89). Dapagliflozin-treated subjects lost on average 3.2 kg, whereas glipizide-treated subjects gained 1.4 kg ($P < 0.0001$). Hypoglycemia (at least one episode over 52 wk) was more frequent

TABLE 3. Effect of dapagliflozin on glycemic control and body weight in T2DM patients

	Dapagliflozin (mg/d)						
	2.5	5	10	20	50	Placebo	Metformin (1500 mg/d)
n	59	58	47	59	56	54	56
Δ HbA_{1c} (%)	−0.71	−0.72	−0.85	−0.55	−0.90	−0.18	−0.73
Δ FPG (mg/dl)	−16	−19	−21	−24	−30	−6	−18
Δ PPG AUC (mg/dl · min)	−9,382	−8,478	−10,149	−7,053	−10,093	−3,182	−5,891
Δ Weight (%)	−2.7	−2.5	−2.7	−3.4	−3.4	−1.2	−1.7

Data are summarized from Ref. 78. FPG, Fasting plasma glucose; PPG AUC, postprandial glucose area under the curve.

in glipizide-treated *vs.* dapagliflozin-treated individuals (40.8 *vs.* 3.5%; $P < 0.001$). Both systolic (−4.3 *vs.* +0.8 mm Hg; $P < 0.001$) and diastolic (−1.6 *vs.* −0.4 mm Hg; $r = 0.02$) blood pressure declined more with dapagliflozin. There were more genital (12 *vs.* 3%) and urinary tract (11 *vs.* 6%) infections in the dapagliflozin group (both $P < 0.05$). There were two cases of pyelonephritis, both in the glipizide group (89).

In a recently completed provocative study, Wilding *et al.* (88) randomized 71 insulin-treated (≥50 U/d) T2DM patients who also were receiving an insulin sensitizer (metformin and/or thiazolidinedione) to add on therapy with dapagliflozin (5 and 10 mg/d) or placebo. The insulin dose was reduced by 50% at the start of therapy, whereas the insulin sensitizer dose was unchanged. After 12 wk of dapagliflozin therapy, the decline in HbA_{1c} was 0.70–0.78% ($P < 0.01$ *vs.* placebo), despite the 50% reduction in insulin dose. Somewhat puzzling, HbA_{1c} was not significantly affected (0.2% increase in HbA_{1c}) by the reduction in insulin dose in the placebo control. The placebo-subtracted reductions in body weight were 2.6 and 2.4 kg, respectively ($P < 0.01$ *vs.* placebo). Both the increase in glucosuria and 50% reduction in insulin dose could have contributed to the weight loss observed in dapagliflozin-treated subjects in this study.

A recent publication compared 151 early-stage (diabetes duration, 1 yr) and 58 late-stage (diabetes duration, 11 yr) T2DM patients who randomly were assigned to 10 or 20 mg/d of dapagliflozin for 12 wk (90). The late-stage diabetic group was in poor glycemic control (HbA_{1c}, 8.4%), was on large doses of insulin (>50 U/d) plus metformin and a thiazolidinedione, and had long-standing diabetes (mean, 11.1 yr) compared with the early-stage group (diabetes duration, 1.0 yr; HbA_{1c}, 7.6%; no antidiabetic medications) (90). The decline in HbA_{1c} was similar in late- and early-stage diabetic patients (Table 4) (90). 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. The greater reduction in body weight in the late-stage diabetic group most likely reflects the reduction in insulin dose because glucose excretion was similar in both groups (Table 4).

In a phase I study, a single dose of sergliflozin (50–500 mg) caused a dose-dependent increase in glucosuria in both normal and T2DM subjects (91, 92). The 500-mg dose reduced the mean plasma glucose concentration during the OGTT from 18.3 to 11.2 mM (91). More prolonged treatment (14 d) with sergliflozin also induced dose-dependent glucosuria with modest weight loss (92). Interestingly, SGLT2 inhibition was accompanied by an increase in plasma glucagon-like peptide-1 concentration and weight loss of 1.5 kg (85).

It is noteworthy that the increase in urine glucose excretion (60–80 g/d) observed with all SGLT2 inhibitors currently in clinical trials represents inhibition of less than 50% of the filtered glucose load. The failure to observe a greater inhibition of renal glucose absorption is unclear but could be explained by: 1) inability of the SGLT2 inhibitor to reach the SGLT2 transporters because of their anatomical location; 2) competitive inhibition that progressively raises the local concentration of glucose at the site of the SGLT2 transporter, thus reducing its effectiveness; 3) insufficiently high drug concentrations in the tubular lumen to inhibit the SGLT2 transporter; 4) in humans, glucose transporters other than SGLT2 may be responsible for a much greater fraction of glucose reabsorption than previously appreciated; and 5) up-regulation of the SGLT1 or other glucose transporters. The latter seems unlikely because the magnitude of glucosuria on d 1–3 *vs.* d 14 after the start of dapagliflozin is similar (83).

Dapagliflozin has not been studied in diabetic patients with reduced GFR. Thus, the efficacy and safety of the SGLT2 inhibitors in diabetic patients with reduced GFR remain to be determined. The filtered glucose load is dependent upon the product of the GFR and the plasma glucose concentration. As the GFR declines, the amount of glucose that is filtered will decrease, thereby reducing glucosuria and glycemic efficacy. The level of GFR at which the efficacy of the SGLT2 inhibitors starts to become impaired and the safety of the SGLT2 inhibitors in diabetic subjects with impaired renal

TABLE 4. Change in HbA_{1c} and body weight in early-stage and late-stage T2DM patients

	ΔHbA1c (%)		ΔWeight (kg)		ΔUrine glucose (g/24 h)	
	Early stage	Late stage	Early stage	Late stage	Early stage	Late stage
Placebo	−0.20	0	−0.95	−1.55	0	−0.8
Dapa 10 mg	−0.70[a]	−0.60[a]	−2.00[a]	−4.30[a,b]	55.0[a]	87.2[a]
Dapa 20 mg	−0.50[a]	−0.80[a]	−2.50[a]	−5.05[a,b]	71.2[a]	87.6[a]

Data are summarized from Ref. 83. Dapa, Dapagliflozin.

[a] $P < 0.05$ *vs.* placebo.

[b] $P < 0.05$ late *vs.* early stage.

function remain to be determined by careful pharmacokinetic/pharmacodynamic studies in this patient group.

It remains to be determined whether the oral SGLT2 inhibitors cause glucosuria by inhibiting the Tm for glucose and/or increasing the glucose splay. One study in rodents with sergliflozin indicates a reduction in Tm without change in the glucose splay (58). We believe that neither of these two explanations (reduced Tm or increased splay) can satisfactorily explain the marked glucosuria induced by the SGLT2 inhibitors in normal glucose-tolerant individuals with a fasting plasma glucose of 5 mM. Rather, we believe that the SGLT2 inhibitors inhibit a constant percentage of the filtered glucose load at all plasma glucose concentrations. At high plasma glucose concentrations, this would result in a greater amount of glucosuria than at low plasma glucose concentrations, although the fractional glucose inhibition would be similar. This does not exclude a concomitant reduction in the glucose Tm and is, in fact, most consistent with the effect of sergliflozin on renal glucose excretion (58).

A. Hyperglycemia and diabetes complications

Hyperglycemia is the principal risk factor for diabetic microvascular complications (retinopathy, nephropathy, and neuropathy) (8, 9). Therefore, improved glycemic control—no matter how achieved—would be expected to reduce the risk of microvascular complications in subjects with T2DM. Because of the important role of enhanced glucose reabsorption in the proximal tubule in altering renal hemodynamics and the development of diabetic nephropathy (93–95), inhibition of renal glucose absorption with a SGLT2 inhibitor might be expected to have an additional beneficial renoprotective action beyond its glucose-lowering effect. The increased filtered glucose load in diabetes results in increased glucose and sodium reabsorption by the SGLT2 transporter in the proximal tubule (35–37). Some investigators (94) have postulated that the primary abnormality resides at the level of the proximal tubule and is characterized by an intrinsic increase in glucose/sodium reabsorption because of a general increase in kidney size and renal (both glomerular and tubular) hypertrophy. In either case, enhanced sodium reabsorption in the proximal tubule leads to a reduction in sodium delivery to the juxtaglomerular apparatus and activates the tubuloglomerular feedback reflex, resulting in vasodilation, elevated intraglomerular pressure, and increased GFR until distal salt delivery returns to its normal set point (96). Renal hyperfiltration and increased kidney size are early characteristic changes of diabetic nephropathy (97) and can be reversed by 6 wk of intensive insulin therapy that normalizes the plasma glucose concentration (97). Therefore, SGLT2 inhibitors could have a dual effect to prevent renal hyperfiltration: 1) normalization of the plasma glucose concentration with reversal of renal hypertrophy, decreased intraglomerular pressure/renal hyperfiltration, and reduced filtered glucose load; and 2) increased sodium delivery to the distal tubule with inhibition of the tubuloglomerular feedback reflex. With regard to this, it is noteworthy that chronic T-1095 administration decreased HbA_{1c} levels in diabetic mice and stopped the progression of diabetic nephropathy with prevention of proteinuria and expansion of glomerular mesangial area (98).

B. Nonglycemic benefits

In addition to the beneficial effects related to improved glycemic control, the SGLT2 inhibitors have a number of nonglycemic effects that make them desirable agents as monotherapy and for combination treatment with other antidiabetic agents. Weight gain is a major problem with currently available antidiabetic medications including sulfonylureas, thiazolidinediones, and insulin. The urinary loss of 60–80 g of glucose per day equates to 240–320 cal/d or 2–3 pounds/month if this caloric deficit is not offset by an increase in caloric intake. Consistent with this, 12–24 wk of treatment with dapagliflozin has been associated with weight losses of 2–3 kg.

A consistent finding in all dapagliflozin studies has been a reduction in blood pressure of 4–5/2–3 mm Hg (78). Although this has been attributed to the mild fluid/sodium deficit that occurs during the first several days of dapagliflozin treatment (83, 86), an equally plausible explanation is local inhibition of the renin-angiotensin system secondary to enhanced sodium delivery to the juxtaglomerular apparatus (94, 95). Consistent with the inhibition of sodium-coupled uric acid reabsorption in the proximal tubule, a consistent decrease in serum uric acid concentration has been observed in diabetic patients treated with dapagliflozin (86). The effect of dapagliflozin on plasma lipid levels has yet to be published (83, 86).

C. Potential side effects

1. Metabolic risks

Hypoglycemia is a potential side effect of all hypoglycemic agents. However, because SGLT2 inhibitors decrease the plasma glucose concentration without augmenting insulin secretion and without inhibiting the counterregulatory response, hypoglycemia is not anticipated with this class of drugs. Indeed, clinical trials have shown that the prevalence of hypoglycemic events in subjects treated with SGLT2 inhibitors was similar to that in people receiving placebo (88–91). Available data from clinical trials of up to 48-wk duration have demonstrated that hypoglycemia was not a significant side effect in this class of drugs. However, when SGLT2 inhibitors are used

in combination with insulin secretagogues and insulin (91), physicians should consider reducing the dose of the sulfonylurea or insulin at the time that SGLT2 therapy is initiated.

2. Renal function

The action of SGLT2 inhibitors on the kidney mimics that observed with osomtic dieresis, and a 400- to 500-ml negative fluid balance occurs during the first 2–3 d of initiating therapy. This modest diuretic action of SGLT2 inhibitors is likely to contribute to the decrease in blood pressure and to explain the small rise in hematocrit (1–2%) and plasma urea nitrogen to creatinine ratio. Importantly, this small increase in urine volume was not associated with a decline in estimated GFR, plasma cystatin concentration, electrolyte disturbances, acid-base balance, hypertension, or the patient's quality of life, *e.g.*, nocturia (88–91).

GFR was not measured during the clinical trials with SGLT2 inhibitors. Because the GFR was not directly measured in these studies, a small decrease in GFR cannot be excluded. Furthermore, these studies were performed in subjects with normal renal function, and the effect of SGLT2 inhibitors on GFR in subjects with impaired renal function remains to be determined. It is also unclear whether the efficacy of the SGLT2 inhibitors will be reduced in diabetic patients with decreased GFR. A decrease in the renal capacity to concentrate the urine is a potential side effect of all agents that causes osmotic diuresis due to washout of the medullary osmotic gradient. The effect of SGLT2 inhibitors on renal concentration capacity has not been examined in clinical studies. Because the diuretic effect of the SGLT2 inhibitors is modest, any dilution of the medullary osmotic gradient and impairment in urine concentrating ability would be anticipated to be modest and without clinical significance, *e.g.*, causes hyponatremia or volume depletion. Of note, tachycardia and orthostatic hypotension (clinical signs of volume depletion) and hyponatremia (laboratory evidence of water depletion) have not been reported in subjects treated with SGLT2 inhibitors. Lastly, it should be emphasized that the current clinical data are from short-term clinical trials (up to 48 wk), and longer follow-up is warranted to establish the long-term safety of SGLT2 inhibitors on renal function.

3. Risk of infection

Because the SGLT2 inhibitors promote glucosuria, they pose a risk for urinary tract infections. Diabetic patients already have significant glucosuria, and it remains to be determined whether the additional glucosuria will promote bacterial growth. In clinical studies, a small (3–5%) increase in the rate of urinary tract infections has been reported in subjects receiving SGLT2 inhibitors compared with placebo (88–90). The majority of these infections involved the lower urinary tract, *i.e.*, cystitis caused by typical urinary tract pathogens, and have responded to standard antibiotic therapy. Because chronic hyperglycemia inhibits phagocytic activity by white blood cells it is possible that any increased risk of bacterial urinary tract infection would be offset by the improved phagocytic activity. The incidence of vulva-vaginitis and balanitis is increased approximately 2-fold in subjects receiving SGLT2 inhibitors (~8–10% *vs.* 3–5% in subjects receiving placebo) (85–92). These fungal infections were reported to subside spontaneously or to respond to local antifungal treatment.

4. Extrarenal effects

Recent studies have reported that the SGLT2 transporter is expressed in extrarenal tissues, liver, and brain. A physiological role for the SGLT2 transporters in liver, heart, and neural tissues has not been identified. However, inhibition of the SGLT2 transporter in these tissues potentially could have a detrimental effect on their function. Abnormalities in liver function tests after SGLT2 treatment have not been observed in clinical studies, and counterregulation in response to hypoglycemia is not inhibited (88–91). Currently, there are no data regarding the ability of SGLT2 inhibitors to cross the blood-brain barrier or on the impact of SGLT2 inhibition on neuronal activity. No central nervous system side effects of SGLT2 inhibitors have been reported in clinical studies, and longer-term studies with specific focus on cognitive function would be of interest to exclude the possibility of central nervous system side effects of SGLT2 inhibitors.

XI. Summary and Conclusion

Current data in experimental animals and humans indicate that inhibition of the SGLT2 transporter is an effective and novel strategy to control the plasma glucose concentration in T2DM subjects. In T2DM patients, dapagliflozin—the most clinically advanced of the SGLT2 inhibitors—has demonstrated a good safety profile, modest weight loss, and HbA_{1c} reduction of approximately 0.7–0.8%, with a starting HbA_{1c} of approximately 8.0%. Because the SGLT2 inhibitors have a distinct mechanism of action that is independent of insulin secretion or the presence of insulin resistance, the efficacy of this class of drugs is not anticipated to decline with progressive β-cell failure or in the presence of severe insulin resistance. Furthermore, this class of drugs can be used in combination with all other antidiabetic medications with anticipated additive efficacy on glycemic control. The SGLT2 inhib-

itors are also effective as monotherapy in newly diagnosed diabetic patients. To the extent that glucotoxicity contributes to the demise in β-cell function in subjects with impaired glucose tolerance or impaired fasting glucose, these drugs also may prove useful in the treatment of "prediabetes." Currently available data indicate that the SGLT2 inhibitors have a good safety profile. However, because this class of drugs is still under development and long-term data are lacking, larger studies with longer follow-up are warranted to establish the long-term safety and efficacy of the SGLT2 inhibitors. In addition, the asymptomatic clinical presentation of subjects with familial renal glucosuria, despite multiple generations of the disease, has documented the long-term safety of pharmacological inhibition of the SGLT2 transporter.

Acknowledgments

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