Renal Sodium-Glucose Cotransporter Inhibition in the Management of Type 2 Diabetes Mellitus

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ABSTRACT

Hyperglycemia is the primary factor responsible for the microvascular, and to lesser extent, macrovascular complications. Despite this well established relationship, approximately half of all type 2 diabetic patients in the US have a HbA1c ≥7.0%. In part, this is associated with the side effects, i.e. weight gain and hypoglycemia, of currently available antidiabetic agents and in part by the failure to utilize medications that reverse the basic pathophysiologic defects present in patients with type 2 diabetes. The kidney has been show to play a central role in the development of hyperglycemia by excessive production of glucose throughout the sleeping hours and enhanced reabsorption of filtered glucose by the renal tubules secondary to an increase in the threshold at which glucose spills into the urine. Recently, a new class of antidiabetic agents, the sodium-glucose cotransporter 2 (SGLT2) inhibitors, has been developed and approved for the treatment of patients with type 2 diabetes. In this review, we examine their mechanism of action, efficacy, safety, and place in the therapeutic armamentarium. Since the SGLT2 inhibitors have a unique mode of action that differs from all other oral and injectable antidiabetic agents, they can be used at all stages of the disease and in combination with all other antidiabetic medications.
Introduction

Hyperglycaemia is *sine qua non* of type 2 diabetes mellitus (T2DM) and is the principal risk factor for the development of diabetic microvascular complications, i.e. retinopathy, nephropathy and neuropathy. T2DM patients also manifest multiple other metabolic abnormalities including obesity, hypertension and dyslipidemia, which complicate management of the diabetic patient and predispose to the development of atherosclerotic cardiovascular complications. The United Kingdom Prospective Diabetes Study (UKPDS) (95) and the Diabetes Control and Complications Trial (DCCT) (24) have demonstrated that for every 1% decrease in hemoglobin A1c (HbA1c) there is a 37% reduction in the risk of microvascular complications. However, despite the irrefutable evidence for the importance of maintaining optimal glycaemic control (HbA1c <6.5–7%), approximately half of people with diabetes in the United States and worldwide fail to achieve this target of glycemic control and manifest a HbA1c >7% (37,86). Weight gain and hypoglycemia, which are encountered with many current antihyperglycemic therapies, are major obstacles that preclude the achievement of the treatment goal in many T2DM patients.

Although lowering the HbA1c decreases the risk of microvascular complications, many clinical trials have demonstrated that tight glycemic control fails to produce a significant decrease in macrovascular events, which are the major cause of death in T2DM patients (4,5,25). Conversely, lowering blood pressure and correcting lipid abnormalities result in robust reductions in macrovascular risk (83). Thus, antihyperglycemic agents that also promote weight loss, lower blood pressure and improve the lipid profile are more likely to have a greater impact on diabetic morbidity and mortality than agents which only lower the plasma glucose concentration.
Inhibitors of renal sodium-glucose cotransport are a novel class of drugs which recently have been approved for the treatment of T2DM (1). In addition to lowering the plasma glucose concentration, members of this class also produce weight loss, lower the blood pressure and are lipid neutral. In addition to lowering the HbA1c, SGLT2 inhibitors have the potential to reduce cardiovascular risk independent of lowering the plasma glucose concentration. In the present review, we will examine the mechanism of action, clinical efficacy and metabolic effects of SGLT2 inhibitors, as well as their place in the management of T2DM.

Renal handling of glucose

The kidney filters approximately 180 liters of plasma each day. Thus, in normal glucose tolerant individuals with a mean day-long plasma glucose concentration of 100 mg/dl approximately 180 grams of glucose are filtered every day. All of this glucose is reabsorbed in the proximal tubule and no glucose is excreted in the urine. Two glucose transporters are responsible for renal glucose reabsorption (100). Both transporters are located in proximal tubule and couple glucose reabsorption to sodium reabsorption (99,100). The sodium-glucose cotransporter 2 (SGLT2) is located in the early (S1) proximal tubule and is responsible for the reabsorption of 80-90% of filtered glucose. SGLT1 is located in the more distal part of the proximal tubule (S2/S3) and is responsible for the reabsorption of the remaining 10-20% of filtered glucose (99,100). The SGLT1 transporter also is expressed in the gut, heart, and lungs (99,100). In the gut, it transports glucose and galactose and is responsible for the absorption of the majority of ingested glucose, as well as galactose. SGLT2 is highly localized to the kidney, thereby minimizing the potential for off target effects. The maximum glucose transport capacity
(Tm) of the proximal tubule, on average, is ~375 mg/min and is slightly higher in men than women (27).

In normal glucose tolerant individuals with a mean day long plasma glucose concentration of ~100 mg/dl, the filtered glucose load (~125 mg/min or 180 grams per day) is less than the maximal renal glucose transport capacity. Thus, all of the filtered glucose is reabsorbed and returned to the circulation. However, as the plasma glucose concentration increases, as occurs in poorly controlled diabetic individuals, the filtered glucose load may exceed 375 mg/min, and all of the filtered glucose in excess of the Tm is excreted in the urine (1,100). The plasma glucose concentration at which the filtered glucose load reaches the Tm (375 mg/min) is called the threshold (Figure 1). The theoretical plasma glucose threshold which corresponds to the Tm (375 mg/min) is ~300 mg/dl. However, in healthy individuals, the plasma glucose threshold at which glucosuria begins is ~180 mg/dL. This difference between the “theoretical threshold” and the “actual threshold” is due to the splay which represents the non-linear transition in the reabsorption and excretion curves as the Tm for glucose is approached. This ‘rounding’ of the curves has been explained by heterogeneity in the Tm of individual nephrons and/or by glomerulotubular imbalance (1,100).

Studies in experimental animals have demonstrated that a chronic increase in plasma glucose concentration, as occurs in diabetes, is associated with up-regulation of SGLT2 expression in the kidney (42). Consistent with this observation, poorly controlled patients with T2DM (27) and T1DM (61) have an increase in the renal Tm for glucose. Moreover, lowering the plasma glucose concentration with insulin has been shown to return the elevated Tm to normal (27), indicating that the chronically elevated plasma glucose concentration is the signal which stimulates the increase in Tm. It has been postulated that the increase in Tm in response to
hyperglycemia developed during the evolution of man to prevent the loss of energy (glucose) during periods of fasting dictated by their hunter-gatherer existence. Today, access to food is not a problem and ~60% of the US population is overweight/obese, thus a mechanism that previously added survival, now has become maladaptive. For both type 2 and type 1 diabetic individuals it would be advantageous for the diabetic kidney to excrete the excess filtered glucose load in an attempt to restore normoglycemia. Unfortunately, the elevated Tm for glucose “minimizes” glucosuria and the hyperglycemia. Consequently, the increased Tm for glucose contributes to the maintenance of hyperglycemia and the kidney should be viewed as an organ that contributes to the pathogenesis of hyperglycaemia in T2DM (19).

Because sodium and glucose are cotransported in renal proximal tubular cells, it is not surprising that type 2 diabetic patients have an increase in total body sodium content and ~60-70% develop hypertension (28). Consistent with this, a 2-3 fold increase in sodium excretion has been demonstrated following acute SGLT2 blockade in diabetic animals. However, renal sodium excretion returned to normal within two weeks, demonstrating the importance of tubuloglomerular feedback in the long-term regulation of salt and water balance (93).

Pharmacological inhibition of renal glucose reabsorption

Phlorizin was the first renal sodium glucose cotransport inhibitor to be identified (17,98). It is a natural compound isolated from the bark of apple trees when they bloom in the spring. Structurally, phlorizin is comprised of a glucose ring, which binds to SGLT, via an oxygen atom (O-glucoside) to two phenol rings. Phlorizin is a nonspecific inhibitor of both SGLT1 and SGLT2 in the proximal tubule. When administered intravenously to healthy individuals phlorizin induces glucosuria, similar to that observed in individuals with familial renal
glucosuria (98), while, in diabetic subjects it causes marked glucosuria and normalizes plasma glucose levels. Low bioavailability (~15%) following oral administration due to gastrointestinal breakdown and inhibition of SGLT1 in the gastrointestinal tract have negated its clinical usefulness in human subjects with diabetes (26).

Substitution of the O-link (between the glucose and phenol moieties) with a C-link provides greater resistance to beta-glucosidase and hence greater bioavailability following oral administration, while substitutions in the phenol rings generate compounds with higher selectivity for SGLT2 compared to SGLT1 and a longer circulating half-life. These structural changes reduce the gastrointestinal side effects observed with phlorizin and make them suitable for single daily dosing. Three members of the SGLT2 inhibitor class (dapagliflozin [Farxiga], canagliflozin [Invokana] and empagliflozin [Jardiance]) have been approved in the US, Europe, and other countries. Non-glucoside inhibitors with even greater SGLT2 selectivity has been developed (53), but have yet to be introduced clinically.

**CLINICAL EFFICACY**

All three approved SGLT2 inhibitors (dapagliflozin, canagliflozin, empagliflozin) produce a dose dependent glucosuria in healthy individuals and in T2DM patients. In healthy individuals, the maximal amount of glucosuria was 62 grams/day with 20 mg dapagliflozin (48), 74 grams/day with 100 mg empagliflozin (33,82) and 70 grams/day with 400 mg canagliflozin (87). Further increases in the drug dose (up to 500 mg dapagliflozin and 800 mg canagliflozin) did not cause any further increase in the amount of glucose excreted in the urine. Similarly, in short term studies (up to 2 weeks), all 3 SGLT2 inhibitors caused a dose dependent increase in urinary glucose excretion (UGE) in T2DM patients and a dose dependent decrease in both the fasting and postprandial plasma glucose concentrations(48,49,87). With more prolonged
treatment (>12 weeks) in T2DM patients, SGLT2 inhibitors produced a dose dependent decrease in HbA1c as monotherapy (2) and in combination with other antidiabetic agents including insulin (7,31,39,76,84,90,101) (Figure 2).

All three approved SGLT2 inhibitors effectively lower the HbA1c in drug naïve T2DM patients. In 24 week studies, the placebo subtracted decrease in HbA1c in T2DM patients with baseline HbA1c ~8.0% was -0.54 and -0.66% for 5 and 10 mg dapagliflozin (n=134) (29), -0.91 and -1.17 for 100 and 300 mg canagliflozin (n=392) (88), and -0.74 and -0.86 for 10 and 25 mg empagliflozin (n=448) (75), respectively.

SGLT2 inhibitors also significantly lower the HbA1c in poorly controlled T2DM patients on other antidiabetic agents. Importantly, the efficacy of SGLT2 in lowering the plasma glucose concentration and HbA1c was independent of the background antidiabetic therapy. Since metformin is the most commonly used antidiabetic agent worldwide, many studies have examined the efficacy of SGLT2 in poorly controlled T2DM patients on metformin monotherapy or metformin plus other antidiabetic agents, e.g. sulfonylureas and thiazolidinediones (TZDs). In a 24 week study with 409 poorly controlled (baseline HbA1c ~8%), metformin-treated T2DM patients, dapagliflozin (5 and 10 mg) caused -0.4 and -0.54% placebo subtracted reduction in T2DM (29). Dapagliflozin has been compared to a sulfonylurea as add-on therapy in metformin-treated T2DM patients (62), with both groups demonstrating a similar decrease in HbA1c (-0.52%) over 52 weeks (Figure 3). However, the time course of decline in the two year groups was quite different. Thus, after six months the HbA1c began to rise progressively in the sulfonylurea-treated group, while it remained stable in the dapagliflozin-treated group, and this trend persisted for up to two years (63).
Likewise, canagliflozin produced a significant reduction in HbA1c in poorly controlled T2DM patients on metformin therapy which was comparable to the decrease in HbA1c caused by sulfonylurea (16) and DPP4 inhibitor (51). In a 52 week study with 1450 T2DM patients 100 and 300 mg canagliflozin caused a -0.82 and -0.93% reduction in HbA1c compared to a -0.81% reduction with sulfonylurea. Similarly, 100 and 300 mg canagliflozin caused a -0.73 and -0.88% reduction in HbA1c over 52 weeks in metformin-treated subjects compared to -0.73 reduction with sitagliptin (5 mg). The baseline HbA1c was ~8.0% in both studies.

Because SGLT2 inhibitors lower the plasma glucose concentration independent of insulin action, they effectively reduce glucose levels in insulin-treated patients. In 71 insulin-treated (≥50 units/day) T2DM patients who also were receiving an insulin sensitizer (metformin and/or thiazolidinedione), dapagliflozin (5 and 10 mg/day) reduced the HbA1c (placebo-subtracted) at 12 weeks by 0.70% and 0.78%, respectively (P < 0.01 vs. placebo) despite a 50% reduction in insulin dose (102). In 800 insulin-treated (~70-80 units/day) type 2 diabetic patients (101) dapagliflozin (2.5, 5 and 10 mg/day) to insulin produced a dose-dependent decline in HbA1c (-0.40, -0.49, and -0.57%, respectively) versus placebo over 48 weeks (101). 100 and 300 mg canagliflozin caused a -0.62 and -0.73% placebo-subtracted reduction in HbA1c in 2072 insulin-treated T2DM patients over 52 weeks (64), while empagliflozin (10 and 25 mg) caused a -0.46 and -0.62% reduction in HbA1c (placebo-subtracted) in insulin-treated T2DM patients (77). Similar reductions in HbA1c have been reported with the addition of empagliflopzin to metformin/sulfonylurea (39) and pioglitazone -treated T2DM subjects (10).

**Durability of SGLT2 Inhibitors**
Studies that have examined the reduction in HbA1c for up to 2 years have demonstrated that SGLT2 inhibitors cause a more durable reduction in HbA1c compared to sulfonylureas (62,63) and DPP4 inhibitors (51,84) (Figure 3). In a head to head comparison between dapagliflozin versus glipizide in 814 poorly controlled T2DM patients on metformin, the reduction from baseline in HbA1c by dapagliflozin and glipizide was identical at 1 year (-0.52%). However, after 2 years the reduction in HbA1c caused by glipizide was attenuated (-0.18%) compared to dapagliflozin (-0.32%) and this trend continued at 4 years (22). Similarly, in a 2-year study, 1450 poorly controlled, metformin-treated T2DM patients (HbA1c=7.8%) were randomized to receive 100 and 300 mg canagliflozin or glimepiride. The maximal reduction in HbA1c with canagliflozin and glimepiride occurred at 12 and 18 months, respectively. Thereafter, the rate of HbA1c increase over time with both doses of canagliflozin was 0.16% per year compared to 0.37% in glimepiride-treated subjects, and at year 2 the reduction in HbA1c with both doses of canagliflozin (-0.58% and -0.60%) was significantly greater than that with glimepiride (-0.38%) (52). Thus, currently available data suggest that SGLT2 inhibitors produce a more durable reduction in HbA1c compared to sulfonylureas and DPP4 inhibitors over a 2-4 year treatment period.

Mechanism of glucosuria

Glucosuria can be induced by lowering the Tm, by reducing the plasma glucose concentration threshold for glucosuria, by increasing the splay, or by a combination of the three. A study in rodents with sergliflozin demonstrated that SGLT2 inhibition markedly reduced the Tm without significant change in the glucose threshold or splay (43). In contrast, in humans dapagliflozin produced a decrease in the Tm as well as a marked reduction in both the threshold
and splay in diabetic and non-diabetic individuals (20) (Figure 4). Using the stepped
hyperglycemic clamp we demonstrated that dapagliflozin decreased the Tm from 420 to 184
mg/min (20). Since, in the presence of dapagliflozin, the Tm is greater than the rate of filtered
glucose in many T2DM patients and occurs at a plasma glucose concentration of ~150 mg/dl
(i.e., well above the fasting plasma glucose concentration in NGT subjects and in many T2DM
subjects), the decrease in Tm alone cannot be sufficient to explain the glucosuria produced by
dapagliflozin. Moreover, dapagliflozin reduced the renal glucose splay in both healthy
individuals and in T2DM patients (Figure 4). Despite the decrease in the splay, dapagliflozin
reduced the threshold of plasma glucose concentration from ~180 mg/dL to ~40 mg/dL (Figure
4). Thus, the decrease in the renal glucose threshold for glucosuria explains the urinary glucose
loss during the fasting state in healthy individuals with fasting plasma glucose concentrations in
the 80-90 mg/dl range.

Although SGLT2 is responsible for the reabsorption of ~80-90% of the filtered glucose
load (~150 grams/24 hours in NGT individuals), the increase in urine glucose excretion (60–70
g/day) observed in NGT individuals with maximal dosages of SGLT2 inhibitors represents the
inhibition of less than 50% of the filtered glucose load (20,33,48,82). This can be explained by
the anatomical location of SGLT1 and SGLT2 in the proximal tubule (99,100) and the glucose
transport capacities of SGLT1 and SGLT2 (3). Because of their proximal location in the S1
segment of the proximal tubule, the filtered glucose first encounters SGLT2 which reabsorbs the
majority (80-90%) of glucose in the glomerular filtrate. Therefore, only a small amount of
glucose reaches the distal part (S2/S3) of the proximal tubule where SGLT1 is located.
Consequently, under physiologic conditions, SGLT1 operates at submaximal transport capacity
(~20%) (Figure 3). However, under conditions of complete inhibition of SGLT2, all of the
filtered glucose reaches the S2/S3 segment of the proximal tubule, and SGLT1 can now use its full capacity (~100-200 grams/day) to reabsorb glucose. Thus, only the fraction of filtered glucose which escapes SGLT1 will be excreted in the urine (Figure 5) (3).

Consistent the preceding scenario, SGLT1 KO mice do not manifest significant glucosuria, documenting that, under non-diabetic conditions, SGLT2 alone is capable of reabsorbing essentially all of the filtered glucose in mice (32,69). However, when SGLT1 KO mice were treated with empagliflozin, virtually all of the filtered glucose was excreted in the urine. These results indicate that the difference between the amount of filtered and excreted glucose during SGLT2 inhibition in normal mice is reabsorbed by SGLT1 (74) (Figure 6).

Further support for this scenario comes from the results of Powell et al (69). In SGLT2 knockout mice approximately 30% of the filtered glucose load appears in the urine. When the SGLT2 knockout mouse was bred with the SGLT1 knockout mouse, urinary glucose excretion increased 3-fold compared with that observed in the SGLT2 knockout mouse (747 versus 224 mg/day). In mice lacking only SGLT1, the amount of glucose excreted in the urine was small (<15 mg/day). These results, in mice, provide conclusive evidence that, in the absence of SGLT2, SGLT1 is capable of reabsorbing ~30% of the filtered glucose load. In man there are no data about SGLT1 expression following chronic SGLT2 inhibition. However, in the SGLT2 knockout mouse there is an ~30% decrease in SGLT1 expression. Studies with the isolated perfused tubule also support an important role for SGLT1 in renal glucose absorption. In the proximal (S1) and distal (S3) parts of the rabbit proximal tubule the glucose transport capacity was reported to be 12.9±1.1 and 7.0±0.5 pmol/min•mm², respectively (8). Immunohistochemical studies have documented the absence of SGLT2 in the distal part of the proximal tubule. Thus, the distal S3 segment of the rabbit proximal tubule that presumably contains only SGLT1 can reabsorb 38%
(7.9/12.9) of the filtered glucose load. These observations are consistent with our estimate in man (3) that the SGLT2 transporter is capable of reabsorbing up to 30-40% of the filtered glucose load under conditions when SGLT2 is inhibited.

When SGLT2 is inhibited, a constant amount of glucose will be reabsorbed by SGLT1 and only the amount of glucose which bypasses SGLT1 will be excreted in the urine. Thus, the fraction of filtered glucose excreted will increase progressively with the increase in the plasma glucose concentration. Further, the amount of glucose removed from the body by SGLT2 inhibition will increase progressively with increasing plasma glucose concentration, as we have demonstrated (20). One would predict that the efficacy of SGLT2 inhibitors in lowering the HbA1c would markedly increase with increasing baseline HbA1c and clinical studies have confirmed this prediction (21,78). In initial exploratory studies, subjects with high initial HbA1c experienced a marked decrease in HbA1c compared to those with lower HbA1c. This point is clearly demonstrated in a clinical study comparing the efficacy of an SGLT2 inhibitor versus a DPP4 inhibitor. In poorly controlled, metformin-treated T2DM patients with an HbA1c <8.0%, the decrease in HbA1c (-0.45%) was slightly lower than that observed with saxagliptin (-0.69%) (Figure 7). However, in T2DM subjects with an HbA1c >9.0% (mean HbA1c= 10.0%) dapagliflozin produced a -1.87% decrease in HbA1c compared to a -1.32% decrease with saxagliptin (78). Stated otherwise, a 2.5% increase in baseline HbA1c from 7.5% to 10% caused a more than 4-fold increase in the efficacy of the SGLT2 inhibitor, while a similar difference in baseline HbA1c caused a less than 2-fold increase in the HbA1c reduction produced by the DPP-4 inhibitor. These results are explained by the greater amount of glucose removed from the body by the SGLT2 inhibitor at higher plasma glucose concentrations and have important clinical relevance (3). It follows that the SGLT2 inhibitor will have a greater advantage in lowering the
HbA1c over other antidiabetic therapies in subjects with a high initial HbA1c (e.g. HbA1c >9.0%). To further put this into perspective, in the study by Ferrannini et al (39), dapagliflozin reduced the HbA1c by 2.7%, from 10.0 to 7.3% in poorly controlled T2DM patients. Thus, combination therapy with dapagliflozin plus another antidiabetic agent can be expected to get the majority of poorly controlled T2DM patients to goal (HbA1c = 6.5-7.0%). This represents a novel approach to the treatment of non-ketotic, poorly controlled T2DM patients and is deserving of future study.

**Dual SGLT1/SGLT2 Inhibitors**

Because SGLT1 is present in the kidney and the gut (99,100), there is concern that inhibition of SGLT1 could be associated with gastrointestinal side effects. Thus, pharmaceutical companies have focused on developing compounds with high selectivity for SGLT2 over SGLT1. However, because under conditions of SGLT2 inhibition, SGLT1 assumes a much greater role in renal glucose reabsorption, there has been renewed interest in a combined SGLT2/SGLT1 inhibitor. Thus, an SGLT2 inhibitor that can partially inhibit SGLT1 would be anticipated to produce significantly greater glucosuria than a highly specific SGLT2 inhibitor. Moreover, because SGLT1 is responsible for absorption of ingested glucose, inhibition of SGLT1 in the gut would ameliorate the rise in postprandial plasma glucose concentration. Further, inhibition of intestinal glucose reabsorption will result in a greater amount of glucose reaching the distal gut where the L-cells are located and stimulate GLP-1 secretion (70). However, since SGLT1 is the sensor in the L-cells for GLP-1 secretion, there is concern that SGLT1 inhibition could impair GLP-1 secretion, and subsequently, insulin release. However, studies in both man (70) and rodents (106) have demonstrated that fermentation products of glucose, i.e. short chain fatty acids, are potent stimulators of GLP-1 secretion and overcome any
effect of SGLT1 inhibition on GLP-1 secretion by the L-cells. Because plasma GLP-1 levels increase during SGLT1 inhibition, this suggests that combination therapy with a DPP-4 inhibitor plus a dual SGLT1/2 inhibitor will have the potential to activate the incretin axis. In one study (106), the dual SGLT1/2 inhibitor, LX4211, caused a small increase in plasma GLP-1 concentration after a glucose load in mice. When LX4211 was combined with sitagliptin, however, a robust increase in plasma GLP-1 was observed following the glucose load. Lastly, SGLT1 protein has been immunolocalized in cardiac capillaries (rather than in previously assumed myocyte sarcolemma) (99). Therefore, clinical development of an SGLT1 inhibitor will require safety studies to exclude a deleterious effect on the myocardium.

**SGLT2 inhibitors and renal function**

Studies with dapagliflozin, canagliflozin, and empagliflozin have demonstrated that SGLT2 inhibition had no clinically significant deleterious effect on renal function (18,20). Although a small decline in eGFR (~4-5 ml/min•1.73m²) has been observed in some studies, the eGFR tended to return to baseline values over time and returned completely to baseline when the SGLT2 inhibitor was discontinued (71). The small decline in eGFR is secondary to the mild reduction in intravascular volume that occurs secondary to inhibition of the sodium-glucose cotransporter and resultant natriuresis (50). The ability of SGLT2 inhibitors to decrease the plasma glucose concentration is strongly related to the level of renal function. With declining GFR, the filtered glucose load is reduced and impairs the drug’s glucose-lowering efficacy. With a GFR = 60–90 mL/min, the glucosuria induced by dapagliflozin (15) was decreased by ~40%, while the HbA₁c declined by only ~22% (Figure 8). In subjects with moderately impaired renal function (GFR = 30–59 mL/min), the glucosuria produced by dapagliflozin (56) and all other SGLT2 inhibitors (46,103) is reduced by ~80% and the decrease in FPG and HbA₁c is quite
modest (4 mg/dL and -0.11%, respectively). In subjects with eGFR < 30 ml/min•1.73m², the
glucosuric effect of the SGLT2 inhibitors is severely impaired and the reduction in HbA1c (0.1-
0.2%) is clinically insignificant (56). The impaired glucosuric effect with declining eGFR is due
to the progressive reduction in the filtered glucose load and the tubular damage that parallels the
glomerular dysfunction. It is noteworthy that, even though the glucose lowering efficacy of the
SGLT2 inhibitors declines with falling eGFR, the blood pressure lowering and weight loss effect
of the SGLT2 inhibitors is largely retained (56).

Metabolic impact of SGLT2 inhibition

Chronic hyperglycemia causes insulin resistance and inhibits insulin secretion, i.e.
glucotoxicity (79). Studies in diabetic animals have demonstrated that normalization of plasma
glucose levels with phlorizin reverse insulin resistance by enhancing GLUT4 translocation and
correct the defects in first- and second-phase insulin secretion (41,80,81). Similarly, in T2DM
humans, we demonstrated that reduction in fasting plasma glucose concentration by 35 mg/dl
with dapagliflozin (10 mg/day for 2 weeks) increased both insulin sensitivity and insulin
secretion (58,59). Since the primary action of dapagliflozin is on the kidney and SGLT2
inhibitors have no direct effect on skeletal muscle, the increase in muscle insulin sensitivity
reflects reversal of glucotoxicity. The improvement in insulin sensitivity was associated with
decreased glucose oxidation and increased lipid oxidation (unpublished results). Similar results
have been reported by others (30). Because increased intramyocellular fat plays a major role in
skeletal muscle insulin resistance (and beta cell failure) (9), we speculate that increased fat
oxidation following SGLT2 inhibition reduces intramyocellular fat content and contributes to
enhanced insulin sensitivity.
Because SGLT2 inhibition lowers the FPG concentration and since the FPG strongly correlates with the basal rate of endogenous (hepatic) glucose production (HGP), we anticipated that SGLT2 inhibition would reduce HGP. Surprisingly, dapagliflozin caused a "paradoxical" increase in basal rate of HGP within 60 minutes after administration and the increase in HGP persisted for the two weeks of dapagliflozin administration (Figure 9) (58). Quantitatively, the amount of glucose added to the circulation by the increase in HGP offset by ~50% the amount of glucose excreted in the urine by SGLT2 inhibition. The increase in HGP was associated with a small decrease in plasma insulin concentration and large increase in plasma glucagon concentration, both of which stimulate HGP (30,58). These observations suggest that addition of an incretin-based agent, i.e. DPP-4 inhibitor or especially a GLP-1 receptor agonist, which simultaneously stimulates insulin and inhibits glucagon secretion, could block the rise in HGP and produce an additive or even a synergistic decrease in plasma glucose concentration and HbA1c. However, recent studies (21,78) have demonstrated that addition of DPP-4 inhibitor to SGLT2 inhibitor failed to produce an additive reduction in plasma glucose concentration despite prevention of the increase in plasma glucagon (34). In subjects with baseline HbA1c >8.5-9.0%, the decrease in HbA1c in subjects treated with DPP-4 inhibitor plus SGLT2 inhibitor was not significantly greater than that caused by the SGLT2 inhibitor alone (78). Although HGP was not measured in these studies, these findings suggest that: (i) the DPP-4 inhibitor failed to prevent the increase in HGP caused by SGLT2 inhibitor and a more potent inhibitor of HGP, i.e. GLP-1 receptor agonist, is required, and (ii) signals other than the increase in plasma glucagon play a key role in mediating the increase in HGP in response to glucosuria. With respect to the latter, the "paradoxical" rise in HGP following dapagliflozin therapy in our studies occurred before the increase in plasma glucagon (58). The rapidity of rise in HGP suggests that activation of renal
nerves may play an important role in mediating the increase in HGP. Lastly, although we believe that the liver is the organ primarily responsible for the increase in EGP following dapagliflozin administration, the kidney cannot be excluded as a contributor.

Lowering the plasma glucose concentration with an SGLT2 inhibitor also improves beta cell function in T2DM patients. Two weeks of dapagliflozin treatment caused a 2-fold increase in beta cell function (59). Similarly, 4-weeks of empagliflozin treatment enhanced beta cell function in T2DM patients (30). These results demonstrate that glucotoxicity plays an important role in the development of beta cell dysfunction (30,59), as well as insulin resistance.

**Hemodynamic impact of SGLT2 Inhibition**

SGLT2 inhibitors block sodium, as well as glucose, reabsorption in the proximal tubule. The inhibition of sodium reabsorption in the proximal tubule during the first 3-4 days of treatment with the SGLT2 inhibitor results in negative sodium balance and a decrease in the extracellular volume (50). T2DM patients, SGLT2 inhibition with dapagliflozin was associated with a 7% decrease in plasma volume which was maintained after 8 weeks (50). The decrease in the extracellular volume most likely is responsible for the reduction in blood pressure (5-6/1-2 mmHg) which is observed within the first 1-2 weeks after initiating therapy with an SGLT2 inhibitor (67).

Inhibition of sodium reabsorption in the proximal tubule by SGLT2 inhibitors also affects intrarenal hemodynamics. Studies in hyperglycemic diabetic rodents have demonstrated increased sodium, with glucose, reabsorption in the proximal tubule (66). This results in decreased sodium delivery to the juxtaglomerular apparatus, a perceived reduction in effective circulating volume, and afferent renal arteriolar vasodilation leading to an elevation in intraglomerular pressure and increase in GFR (hyperfiltration) which play an important role in
the development of diabetic nephropathy (66). By inhibiting sodium, along with glucose, transport in the proximal tubule and increasing sodium delivery to the juxtaglomerular apparatus, SGLT2 inhibitors, cause afferent renal arteriolar vasoconstriction, decreased intraglomerular pressure and reduction in GFR hyperfiltration. Studies in diabetic mice have demonstrated that chronic SGLT2 inhibition with T-1095 decreased HbA1c and stopped the progression of diabetic nephropathy with prevention of proteinuria and normalization of glomerular mesangial area (6). Consistent with the preceding scenario, hyperfiltration and increased kidney size in type 1 diabetic patients can be reversed by 6 weeks of intensive insulin therapy that normalizes the plasma glucose concentration (94). A recent study demonstrated that 8 weeks of treatment with empagliflozin also reversed hyperfiltration and decreased intraglomerular pressure in poorly controlled type 1 diabetic patients due to afferent renal arteriolar vasoconstriction (18). Importantly, no active comparator group was included in this study. Therefore, it is difficult to determine whether the reversal of the hyperfiltration was due to reduction in plasma glucose concentration or increase in sodium delivery to the juxtaglomerular apparatus or some combination of the two. Interestingly, empagliflozin had no significant effect on GFR in T1DM without hyperfiltration. Thus, whether SGLT2 inhibitors exert a renoprotective action independent of their glucose lowering effect remains to be determined.

**Weight loss**

The majority of T2DM patients are overweight or obese, and many of the currently available antidiabetic medications, e.g. sulfonylureas, thiazolidinediones, and insulin, are associated with weight gain. The urinary loss of 60–80 grams/day of glucose (4 calories/gram) equates to 240–320 cal/day or ~2–3 pounds per month. Consistent with this, weight loss has been observed in diabetic subjects treated with SGLT2 inhibitors in all clinical studies.
However, body weight tends to level off within 6 months after the start of SGLT2 inhibitor therapy (7,16,29,31,35,39,51,62-64,75-77,84,88,90,101,102), suggesting a compensatory increase in food intake. Indeed studies in experimental animals have demonstrated an increase in food intake following treatment with SGLT2 inhibitors (23). This raises the intriguing possibility that combination therapy with an SGLT2 inhibitor plus a GLP-1 receptor agonist or other appetite suppressant drug could produce an additive, even synergistic, effect to promote weight loss.

**Effect of SGLT2 inhibitors on plasma lipid profile**

In phase III trials, canagliflozin produced a small, but significant, increase (~8%) in plasma HDL cholesterol concentration and a significant decrease (~5%) in plasma triglyceride concentration in drug naive subjects and in T2DM patients poorly controlled with metformin (11,89). Canagliflozin also caused a small, but significant, increase (~5%) in plasma LDL cholesterol levels, which was independent of background statin therapy (11,89). Although the increase in plasma HDL and decrease in plasma triglyceride concentrations could be explained by the weight loss (9), the etiology of the increase in LDL cholesterol is unknown. Although the increase in LDL cholesterol is small, its impact on cardiovascular events is unknown and awaits the completion of CANVAS, which includes 4,300 high risk T2DM subjects and is due to be read out in 2017 (64). Similar effects on the plasma lipid profile have been observed with dapagliflozin and empagliflozin and long term cardiovascular safety studies with empagliflozin (EMPA-REG) and dapagliflozin (DECLARE) are ongoing (73).

**Effect on blood pressure:** The salt and water deficit that occurs during the first several days of SGLT2 treatment and the weight loss that occurs with more long term therapy (50)
contribute to the decrease in blood pressure. In all clinical trials with SGLT2 inhibitors, reduction in systolic/diastolic blood pressure of 5-6/1-2 mmHg has been a consistent finding (67). Local inhibition of the renin-angiotensin system secondary to enhanced sodium delivery to the juxtaglomerular apparatus (93,96) can provide an alternative explanation for the decrease in blood pressure. In T2DM patients treated with PF04791729, the blood pressure reduction was similar to that produced with thiazide diuretics. Uric acid reabsorption is coupled to sodium reabsorption in the proximal tubule. SGLT2 inhibitors block sodium reabsorption, and with it uric acid reabsorption, in the proximal tubule. This leads to an increase in uric acid excretion and resultant decrease in serum uric acid concentration of 0.8-1.0 mg/dl (102).

Safety

Because of their high selectivity for SGLT2 over SGLT1, significant inhibition of gut glucose/galactose transport does not occur and gastrointestinal side effects have not been observed. The major side effect observed with SGLT2 inhibitors is mycotic vaginal infections in females, occurring in ~1.5-2% of placebo-treated subjects and 7-8% of women treated with an SGLT2 inhibitor (7,16,29,31,35,39,51,62-64,73-75,88,90,101,102). A small increase in balanitis has been observed in males, primarily in men who are uncircumsized (7,16,29,31,35,39,51,62-64,73-75,88,90,101,102). Although the package insert for dapagliflozin, canagliflozin, and empagliflozin states that the incidence of urinary tract infections is increased in patients treated with an SGLT2 inhibitor, this is not substantiated by the results of clinical trials (7,16,29,31,35,39,51,62-64,73-75,88,90,101,102) or by the information provided in the package inserts. Diabetic patients in poor control and are prone to develop UTIs (45). In one study in
which mid-stream urine was collected, treatment with an SGLT2 inhibitor did not increase the prevalence of urinary bacteriuria (40).

An increased incidence of volume-related side effects (patient reported symptoms) has been demonstrated in diabetic patients receiving treatment with SGLT2 inhibitors, especially elderly individuals and individuals treated with diuretics (97). However, documentation of orthostatic hypotension by direct blood pressure measurement was not employed in these studies.

In diabetic patients treated with dapagliflozin for 12-24 weeks, a small increase in urine volume and sodium excretion was observed during the initial 2-3 days of treatment (55), and this was accompanied by a small rise in hematocrit (1–2 volume %) and plasma urea nitrogen to creatinine ratio. Plasma concentrations of Na\(^+\), K\(^+\), Cl\(^-\), Ca\(^{++}\) remained unchanged following dapagliflozin treatment (55,97). An increase in plasma potassium concentration rarely has been reported with canagliflozin (60), but it is unclear whether this is related to the SGLT2 inhibitor per se or to the unrecognized presence of the hyporeninemic hypoaldosteronemia syndrome (54).

Hyperkalemia has not been reported with other SGLT2 inhibitors. Changes in plasma sodium, chloride, calcium, and bicarbonate concentrations have not been observed with the SGLT2 inhibitors.

As previously reviewed, SGLT2 inhibitors do not adversely affect renal function or cause/exacerbate albuminuria in patients with both T2DM (18,20). To the contrary, a decrease in proteinuria has been reported with SGLT2 inhibitors in experimental animals (46,97) and humans (72,104), and the decrease was independent of lowering the plasma glucose concentration. Importantly, subjects with mutations in the SGLT2 gene maintain normal kidney function without proteinuria despite excreting large amounts of glucose (>50–100 g/24 h) over a
lifetime (85). Moreover, some of these individuals with familial renal glucosuria have had renal biopsies which revealed completely normal renal histology (105).

Although hypoglycaemia is a potential concern with all antidiabetic agents, when used as monotherapy or with agents other than sulfonylureas or insulin, no increase in the incidence of hypoglycemia has been observed with SGLT2 inhibitors, even when given to normoglycemic individuals (105). This can be explained by an increase in glucagon secretion (58) and the stimulation of HGP.

An increase in the incidence of bladder and breast cancer was observed in the dapagliflozin clinical trials, but the total number of cases was small. Of note, SGLT2 is not expressed in either breast or bladder tissues, and carcinogenic studies in multiple animal species did not detect any preneoplastic or neoplastic activity. Breast, and particularly bladder, cancer are known to take many years to develop. In the clinical trials dapagliflozin exposure was short (less than one year). Therefore, it seems unlikely that the small increase in the incidence of these two tumors is related to the SGLT2 inhibitor. Since frequent testing for UTIs was performed in these clinical trials, this could have introduced detection bias for bladder cancer by leading to the discovery of microscopic hematuria. Further, 7 of the 10 cases of bladder cancer had hematuria at the time of entry into the study. Nonetheless, because of this potential carcinogenic signal, the producer (Astra Zeneca) of dapagliflozin has committed to a long-term surveillance study. No increase in the incidence of bladder cancer has been observed with either canagliflozin or empagliflozin.

Recent reports have demonstrated the development of ketoacidosis in diabetic patients treated with SGLT2i (38,68,91). Studies by Ferrannini et al (30), as well as by Merovci et al (58), have shown that type 2 diabetic subjects treated with SGLT2 inhibitors demonstrate a
decrease in glucose oxidation and a reciprocal increase in fat oxidation. We interpret these changes in substrate oxidation as follows. In T2DM individuals muscle tissue is severely resistant to insulin (19). Consequently, plasma glucose concentration rises to a level that is sufficient to augment glucose entry into the cell by the mass action effect of hyperglycemia (12).

Treatment with SGLT2 inhibitors induce glucosuria, causing an acute reduction in plasma glucose concentration and decreased glucose entry into muscle both in postabsorptive and insulin-stimulated state. In order to meet the energy demand of the cell, myocytes and hepatocytes switch to fat as an alternate source of energy. Increased fat oxidation results in increased production of acetyl-CoA, which either can be oxidized in the Krebs cycle or converted to ketones (acetoacetic and beta hydroxybutyric acid). The plasma glucagon: insulin ratio perfusing the liver is a key determinant of the fate of acetyl-CoA (57). Glucagon stimulates the expression of hydroxyl-methylglutaryl-CoA synthase (HMGS), the rate limiting step for the conversion of acetyl-CoA to ketones, while insulin suppresses its expression. The increase in fasting plasma glucagon concentration observed in T2DM individuals treated with SGLT2i (13,30,58) would be expected to increase HMGS activity, causing an increase in ketone production. Clinical reports documenting the development of ketoacidosis following the initiation of SGLT2i therapy in diabetic patients (38,68,91) share a number of features in common. Many cases involved type 1 diabetic patients in whom the insulin dose was reduced when SGLT2i therapy was initiated. Since SGLT2i increase glucagon secretion (13,30,58), it is not surprising that insulin dose reduction might result in ketoacidosis. Another common feature in many cases was an associated medical or surgical condition resulting in moderate-severe stress. Release of catecholamines predisposes to the development of ketoacidosis both by inhibiting insulin secretion and stimulating ketone production (44).
The place of SGLT2 inhibitors in the management of T2DM individuals

The American Diabetes Association/European Association for the Study of Diabetes recommends metformin as the first-line therapy in individuals with new-onset type 2 diabetes. However, because metformin does not affect beta-cell function, metformin-treated individuals experience a progressive increase in HbA1c after an initial good response (14,95). SGLT2 inhibitors provide a therapeutic option in metformin-failing diabetic individuals or in individuals who cannot tolerate metformin because of adverse gastrointestinal side effects. Moreover, because of their unique mechanism of action on the kidney, the SGLT2 inhibitors effectively can be used in combination with all other antihyperglycemic agents including insulin. Further, they promote weight loss, reduce blood pressure, and have an advantage over other antidiabetic agents in subjects with very high HbA1c, e.g. HbA1c >9%. This later group of patients often is treated with insulin to correct the metabolic decompensation, i.e., glucotoxicity and lipotoxicity. In this group of individuals metformin alone will not lower the HbA1c to the treatment goal (<6.5-7.0%). In a study of new-onset T2DM patients initiation of therapy with metformin plus dapagliflozin produced an additive decrease in HbA1c versus either therapy alone, and more subjects (~60%) with the combination therapy achieved the target glycemic goal (HbA1c <7.0%) than with either therapy alone (36). Because SGLT2 inhibitors are effective in lowering the HbA1c at all stages of diabetes, they can be added in subjects who are inadequately controlled with multiple oral agents, GLP-1 receptor agonists and/or insulin therapy. Because of their potential to produce an additive or even synergistic effect to reduce the HbA1c and promote weight loss, combination therapy with an SGLT2 inhibitor and GLP-1 receptor agonist may be an especially effective intervention. Lastly, SGLT2 inhibitors also can be used in combination
with basal insulin therapy to improve glycemic control while promoting weight loss and reducing the dose of insulin without provoking hypoglycemia (101).

Lastly, the efficacy of the SGLT2 inhibitors quickly can accessed by measuring: (i) the decline in fasting plasma glucose concentration in the morning following the first administration of the SGLT2 inhibitor or (ii) the fractional excretion of glucose \((U_{\text{glu}} \cdot P_{Cr}/U_{Cr} \cdot P_{\text{glu}})\) following the first dose administration. A fractional excretion >30-40% indicates a good therapeutic response even in those with a reduced GFR.
Conflict of interest statement

RAD is a member of the Advisory Board of Janssen, Boehringer Ingelheim, Astra Zeneca, Novo Nordisk, Intarcia and Lexicon. RAD is a member of the Speaker Bureau of Novo Nordisk and Astra Zeneca. RAD has grant support from Astra Zeneca, Janssen, and Boehringer Ingelheim.

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FIGURE LEGENDS

Figure 1. Kinetics of renal glucose handling.

Figure 2. Effect of background therapy on A1c reduction in type 2 diabetic patients treated with canagliflozin, dapagliflozin, and empagliflozin. Drawn from the data in references #2,7,29,31,35,39,76,84,90,101.

Figure 3. Time course of effect of dapagliflozin (n=400) versus glipizide on the decrement in A1c in metformin-treated type 2 diabetic patients (left panel). Redrawn from ref #22 and 62. Time course of effect of canagliflozin (n=377) versus sitagliptin (n=378) in poorly controlled type 2 diabetic patients treated with metformin plus sulfonylurea (right panel). Redrawn from ref #84.

Figure 4. Effect of dapagliflozin on the renal Tmg (top), threshold (middle), and splay (bottom) for glucose in healthy normal-glucose-tolerant and type 2 diabetic subjects. From ref #20.

Figure 5. Renal tubular reabsorption of glucose in healthy normal-glucose-tolerant subjects with a GFR of 180 L/day and a mean day-long plasma glucose concentration of 100 mg/dl (left panel). Impact of complete SGLT2 inhibition on glucose reabsorption by SGLT1 and glucose excretion. Adapted from ref #3.

Figure 6. Renal tubular glucose reabsorption in wild type, SGLT1 knockout and SGLT2 knock out mice (left panel). Renal tubular reabsorption in wild type mice and SGLT1 knockout mice treated with empagliflozin. From ref #74.

Figure 7. Influence of baseline HbA1c on the efficacy of dapagliflozin and saxagliptin. From ref #78.
Figure 8. Impact of reduced renal function on the glucose-lowering efficacy (A1c) of dapagliflozin (adapted from Bristol Myers Squibb NDA; ref #15).

Figure 9. Effect of dapagliflozin on endogenous (primarily reflects hepatic) glucose production in type 2 diabetic subjects. The first two symbols on the curves represent the basal rate of EGP. Dapagliflozin or placebo was administered at 9AM on day 1. From ref #58.
Figure 3

- Dapagliflozin (BI A1c = 7.69%)
- Glipizide (BI A1c = 7.74%)
- SITA 100 mg (BI A1c = 8.1%)
- CANA 300 mg (BI A1c = 8.1%)

Week 5

Weeks
Figure 5

(180 L/day) (1000 mg/L) = 180 g/day

Glucose

SGLT2

SGLT1

S2/S3

0 g/d

~120 g/d, 100% Occupancy

~60 g/d
Figure 6

Fraction of Filtered Glucose Reabsorbed (%)

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Empagliflozin

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Figure 7

Baseline A1c (%)  
<8.0  >9.0

Mean A1c (%)  
7.5  10.0

Change in A1c (%)  
-0.45%  -0.69%  -1.87%  -1.32%