Renal, metabolic and cardiovascular considerations of SGLT2 inhibition

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Abstract | The kidney has a pivotal role in maintaining glucose homeostasis by using glucose as a metabolic fuel, by producing glucose through gluconeogenesis, and by reabsorbing all filtered glucose through the sodium–glucose cotransporters SGLT1 and SGLT2 located in the proximal tubule. In patients with diabetes, the maximum glucose reabsorptive capacity (Tm_G) of the kidney, as well as the threshold for glucose spillage into the urine, are elevated, contributing to the pathogenesis of hyperglycaemia. By reducing the Tm_G and, more importantly, the threshold of glucosuria, SGLT2 inhibitors enhance glucose excretion, leading to a reduction in fasting and postprandial plasma glucose levels and improvements in both insulin secretion and insulin sensitivity. The beneficial effects of SGLT2 inhibition extend beyond glycaemic control, however, with new studies demonstrating that inhibition of renal glucose reabsorption reduces blood pressure, ameliorates glucotoxicity and induces haemodynamic effects that lead to improved cardiovascular and renal outcomes in patients with type 2 diabetes mellitus. In this Review we examine the role of SGLT2 and SGLT1 in the regulation of renal glucose reabsorption in health and disease and the effect of SGLT2 inhibition on renal function, glucose homeostasis, and cardiovascular disease.

Type 1 and type 2 diabetes mellitus are systemic cardiometabolic diseases that affect both the microvasculature and macrovasculature. They are characterized by hyperglycaemia, which is a major risk factor for microvascular complications, including nephropathy, retinopathy and neuropathy[1,2]. By contrast, hyperglycaemia is a relatively weak risk factor for macrovascular complications, such as myocardial infarction, stroke and peripheral vascular disease[1], and any meaningful benefit of improved glycaemic control on macrovascular complications can take up to 10 years to manifest[3,4]. The major risk factors for macrovascular complications are dyslipidaemia, hypertension, obesity, insulin resistance and a prothrombotic state[5,6]. Before 2015, neither glucose lowering medications[7–9] nor lifestyle intervention[10] had been shown to reduce macrovascular complications in patients with type 2 diabetes mellitus (T2DM). In the past ~14 months, however, two classes of antidiabetic drugs — sodium–glucose cotransporter 2 (SGLT2) inhibitors and glucagon-like peptide 1 (GLP-1) receptor agonists — were shown to reduce major cardiovascular end points in trials of patients with T2DM[11,12]. Moreover, SGLT2 inhibitors were associated with slower progression of kidney disease and dramatically reduced rates of clinically relevant renal end points[13]. SGLT2 inhibitors are unique as they target the transport of glucose in the kidney, preventing glucose reabsorption and inducing glucosuria to lower plasma glucose levels. In the present Review we examine the role of the kidney in maintaining glucose homeostasis, and the effect of SGLT2 inhibition on metabolic, cardiovascular, and renal function in healthy individuals and in patients with T2DM.

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Glucose metabolism in the kidney

The rate of endogenous glucose production following an overnight fast is ~2 mg/kg per minute and closely matches the rate of glucose uptake by all tissues in the body[14]. Approximately 80% of endogenous glucose is produced by the liver and the remaining ~20% by the kidney[15] (FIG. 1). Insulin is a powerful inhibitor of hepatic and renal glucose production[15]. The kidney contains the necessary gluconeogenic enzymes to produce glucose. Unlike glucagon, which stimulates hepatic but not renal glucose production[16,17], adrenaline does stimulate renal glucose production[16,17]. The liver and kidney also differ in their gluconeogenic amino acids: glutamine is the major gluconeogenic amino acid in the kidney, whereas alanine is the primary gluconeogenic amino acid in the liver[15] (FIG. 1).

Glucose produced by the kidney is primarily derived from renal cortical cells[13], whereas glucose utilization within the kidney is primarily by renal medullary cells[15].

Maximum renal glucose reabsorptive capacity (Tm_G). The Tm_G represents the maximum capacity of the renal tubule to reabsorb glucose that is filtered by the glomerulus, and is expressed in mg per minute.

Key points

- The kidneys contribute to the maintenance of normal glucose homeostasis by using glucose as a metabolic fuel, by producing glucose via gluconeogenesis, and by reabsorbing all filtered glucose
- Under physiological conditions SGLT2 reabsorbs the majority (80–90%) of filtered glucose, while SGLT1 reabsorbs the remaining 10–20% of glucose
- Kidneys contribute to the development of hyperglycaemia in diabetes by producing excess amounts of glucose and by increasing glucose reabsorption in response to an elevated threshold for glucosuria and an increase in the maximum glucose reabsorptive capacity (Tm_G)
- SGLT2 inhibitors improve glucose tolerance by reducing both the threshold for glucosuria and the Tm_G and by ameliorating glucotoxicity leading to enhanced β-cell function and improved insulin sensitivity in muscle
- The efficacy of SGLT2 inhibitors is partially offset by an increase in endogenous glucose production and enhanced glucose reabsorption by SGLT1
- Findings from the EMPA-REG OUTCOME study suggest that the SGLT2 inhibitors might be beneficial in reducing cardiovascular events and preventing the progression of renal disease in patients with type 2 diabetes mellitus at high cardiovascular risk

Consequently, the net arteriovenous balance of glucose across the kidney is zero[15,17]. In individuals with T2DM the basal rates of renal and hepatic glucose production are increased despite elevated fasting plasma insulin levels, indicating a state of hepatic and renal insulin resistance[14].

Currently, little is known about the effect of SGLT2 inhibitors on renal glucose production or uptake in humans. Gluconeogenesis from glutamine generates ammonia (NH_3), which facilitates hydrogen ion excretion (in the form of $NH4^+$) in response to metabolic acidosis. In rodents SGLT2 inhibitors reduce the activity of phosphoenolpyruvate carboxykinase (PEPCK)[18], a key gluconeogenic enzyme. Patients with T2DM treated with SGLT2 inhibitors have been reported to develop ketoacidosis[19], which might arise from inhibition of PEPCK leading to impaired ammonia production.

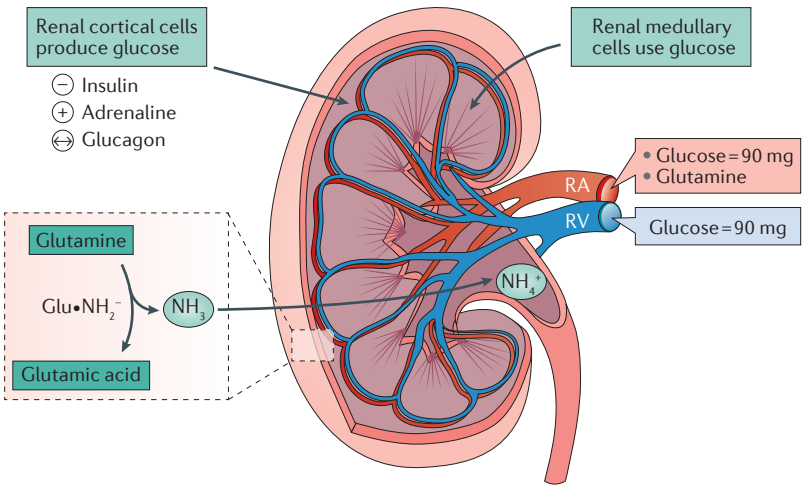

Figure 1 | **Role of the kidney in glucose homeostasis.** The kidney utilizes glucose as an energy source (renal medullary cells) and produces glucose via gluconeogenesis (renal cortical cells). The net result is that the net arteriovenous balance of glucose across the kidney is zero. Glucose production by the renal cortical cells is inhibited by insulin and stimulated by adrenaline. Glucagon has no effect on renal glucose production. Glutamine is extracted by renal tubular cells and used to generate ammonia (NH_3), which has an important role in acid excretion (NH_4^+). RA, renal artery; RV, renal vein.

The kidney in glucose homeostasis

SGLT1 and SGLT2

In normal glucose-tolerant individuals with a glomerular filtration of ~180 l per day and mean day-long plasma glucose concentration of ~5.6 mmol/l (~100 mg/dl), the kidney filters ~180 g glucose daily (FIG. 2). All of the filtered glucose is reabsorbed in the early proximal tubule and in nondiabetic individuals no glucose appears in the urine. The majority of filtered glucose (80–90%) is reabsorbed by the SGLT2 transporter in the early S1 segment of proximal tubule[20–23], while the remaining 10–20% is reabsorbed by the SGLT1 transporter in the more distal S2/S3 segment (FIG. 2). More than 90% of the filtered glucose is reabsorbed within the initial 4 mm of the proximal tubule from the glomerulus. Although SGLT2 transporters are primarily located in the kidney, they are also found in pancreatic α cells[22] and other tissues, such as the cerebellum[21]. SGLT2 inhibition in the pancreatic α cell stimulates glucagon secretion[22], which antagonizes the glucose-lowering effect of SGLT2 inhibitors by augmenting hepatic glucose production[24]. The only study that has examined the effect of glucagon on renal glucose production in humans found no effect[17], but this observation awaits confirmation. The function of SGLT2 transporters in other tissues, including the cerebellum, is unknown.

The SGLT1 transporter is more widely distributed being expressed in gut, heart and lungs[20,21]. SGLT1 is the primary transporter responsible for glucose absorption in the intestine, and intestinal inhibition of SGLT1 causes glucose–galactose malabsorption. In the kidney, glucose reabsorption by SGLT1 accounts for only 10–20% of the filtered glucose load because of its more distal location; however, considerable evidence indicates that SGLT1 does not act at full capacity under physiologic conditions, as discussed in further detail later.

Glucose reabsorptive capacity

In humans the maximum renal glucose reabsorptive capacity (Tm_G) is ~375 mg per minute, being slightly greater in men than in women[25,26]. In normal glucose tolerant individuals the rate at which glucose is filtered (~180 g per day or ~125 mg per minute) is considerably

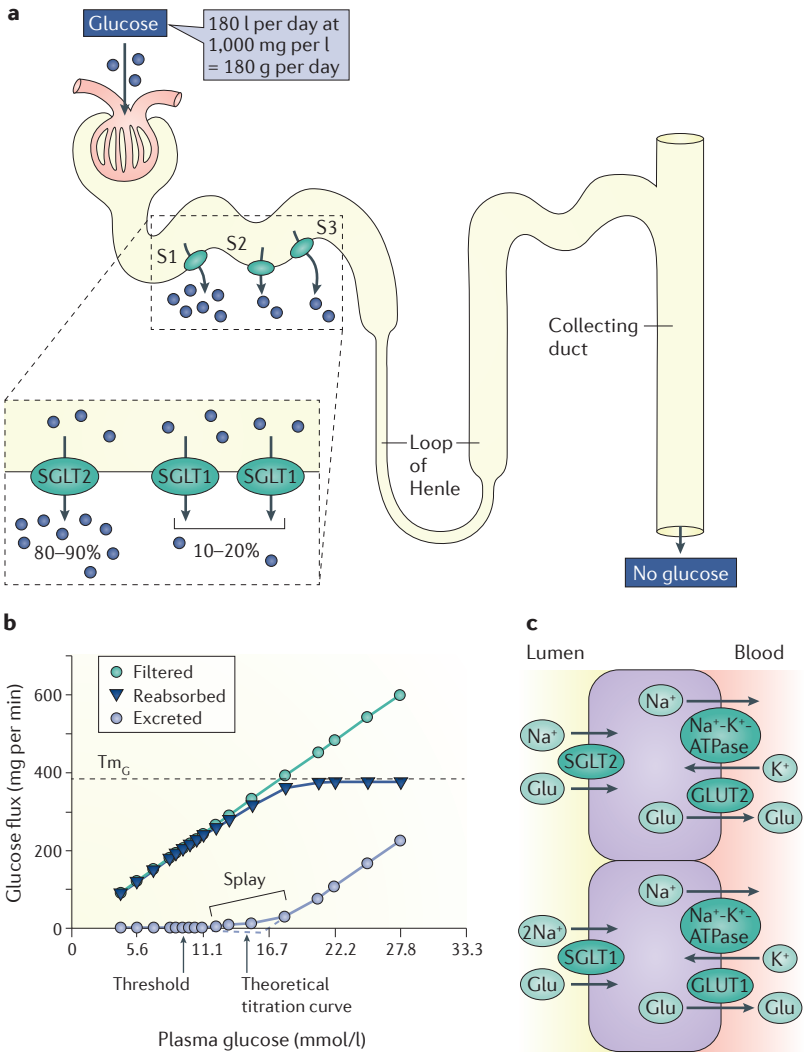

Figure 2 | **Glucose handling by the kidney. a** | Normal glomerular filtration rate is ~180 l per day. With a mean day-long plasma glucose concentration of ~5.6 mmol/l (100 mg/dl), the kidney filters ~180 g of glucose every day, yet no glucose appears in the urine. Under physiological conditions, the sodium–glucose cotransporter 2 (SGLT2) in the S1 segment of the proximal tubule reabsorbs 80–90% of the filtered glucose while the remaining 10–20% is reabsorbed by SGLT1 in the S2/S3 segment. **b** | Kinetics of renal glucose reabsorption. In healthy glucose tolerant individuals, the maximum renal tubular glucose reabsorptive capacity (Tm_G) is ~375 mg per minute, which is considerably greater than the rate at which glucose is filtered (125 mg per minute). The point at which the plasma glucose concentration exceeds ~10 mmol/l or ~180 mg/dl is referred to as the threshold for glucosuria, above which all of the excess filtered glucose is excreted in the urine. The 'actual threshold' for glucose spillage in the urine is lower than the 'theoretical threshold'. The difference between the two thresholds is referred to as splay and characterizes the nonlinear transition between the renal glucose reabsorption and excretion curves as the Tm_G is approached. **c** | The energy for sodium–glucose transport (through both SGLT2 and SGLT1) is derived from the Na^+-K^+-ATPase pump that is located in the basolateral membrane of the proximal tubule. This pump drives sodium (Na^+) out of the cell in exchange for potassium (K^+). As the intracellular sodium concentration declines, sodium moves passively with glucose (Glu) from the tubular lumen to the intracellular domain via the SGLT transporters. Once within the proximal tubular cell, glucose moves passively down its concentration gradient into the interstitial space via the GLUT transporters. SGLT2 and GLUT2 represent a coupled transport mechanism, as do SGLT1 and GLUT1. Parts **a**,**c** are modified with permission from Abdul-Ghani, M. A. *et al.* Role of sodium glucose cotransporter 2 (SGLT2) inhibitors in the treatment of type 2 diabetes. *Endocrine Rev.* **32,** 515–531 (2011); permission conveyed through Copyright Clearance Center, Inc.

less than the Tm_G and all filtered glucose is reabsorbed in the early proximal tubule with none appearing in the urine. In patients with poorly controlled T1DM or T2DM, however, the filtered glucose load can exceed the threshold, resulting in glucosuria. In healthy, non-diabetic individuals, no glucose appears in the urine until the plasma glucose concentration exceeds ~10 mmol/l (180 mg/dl), which is referred to as the threshold for glucosuria (FIG. 2b). However, the theoretical threshold, corresponding to a Tm_G of 375 mg per minute, is actually ~17 mmol/l (300 mg/dl). The difference between the 'theoretical' threshold and 'actual' threshold is referred to as splay and characterizes the nonlinear transition between the renal glucose reabsorption and excretion curves as the Tm_G is approached (FIG. 2b). Splay has been explained by the presence of either functional glomerulotubular imbalance (such that tubular reabsorption is not in balance with the rate of single nephron glomerular filtration) and/or morphological glomerulotubular imbalance (with heterogeneity between individual nephrons resulting in differences in their ability to reabsorb glucose). Thus, glucosuria can result from a reduction in Tm_G, a decrease in the threshold for glucosuria, or an increase in splay.

Glucose reabsorption in hyperglycaemia

Patients with poorly controlled type 1 diabetes mellitus (T1DM) and T2DM[25,27], and experimental models of diabetes[27,28] have increased Tm_G compared to that of nondiabetic subjects, which can return towards normal following normalization of plasma glucose levels with intensive insulin therapy[25]. Our studies[26,29] have shown that the renal threshold for glucosuria, as well as the Tm_G, are elevated in patients with T2DM, even in those with relatively good glycaemic control (glycated haemoglobin (HbA_{1c}) levels ~6.5%), and increases progressively with increasing HbA_{1c} levels. In cultured proximal renal tubular cells from patients with T2DM, levels of SGLT2 mRNA and protein, and the intrinsic transport capacity of SGLT2 for glucose, were increased[30], although the specificity of the antibody used to determine protein levels and the purity of the proximal renal tubular preparation used in this study could be questioned. Increased *SGLT2* mRNA also has been demonstrated in the kidney of animals with experimentally induced diabetes[18,31–33]. However, we have found normal-to-reduced *SGLT2* mRNA levels and markedly increased *SGLT1* mRNA levels in patients with T2DM compared to nondiabetic individuals (L. Norton, unpublished work) . The increase in threshold and Tm_G in response to hyperglycaemia most likely represents an evolutionary adaptation to prevent glucose loss and conserve energy during conditions of famine. Today, when food is abundant and T2DM is epidemic, this adaptive process is maladaptive, and the increases in threshold for glucosuria and Tm_G that occur with increasing HbA_{1c} levels represent important pathophysiologic abnormalities that contribute to the maintenance of hyperglycaemia in patients with diabetes[14,26].

The active step that mediates sodium and glucose resabsorption in the proximal tubule involves the Na^+-K^+-ATPase pump, which is located on the basolateral

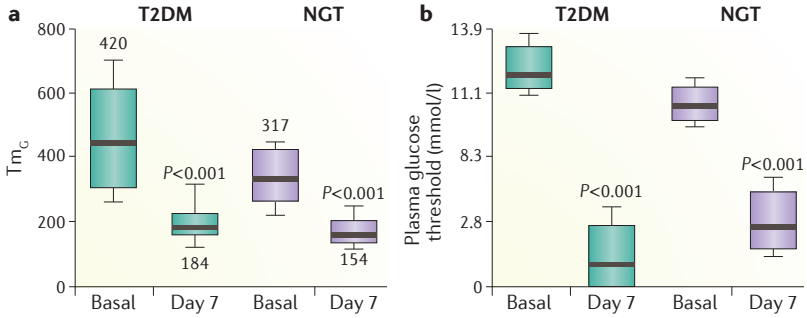

Figure 3 | **Effect of dapagliflozin on maximum renal glucose reabsorption (Tm_G) and glucose threshold. a** | In individuals with type 2 diabetes mellitus (T2DM) the Tm_G is significantly increased in normal glucose tolerant (NGT) individuals and similarly reduced by dapagliflozin in both groups. **b** | In patients with T2DM the renal threshold for glucose spillage is significantly increased compared to NGT individuals and reduced to less than 2.2 mmol/l (40 mg/dl) in both groups following dapagliflozin treatment. This effect explains why SGLT2 inhibitors produce marked glucosuria in non-diabetic individuals with a normal fasting plasma glucose concentration. Modified with permission from DeFronzo, R. A. *et al.* Characterization of renal glucose reabsorption in response to dapagliflozin in healthy subjects and subjects with type 2 diabetes. *Diabetes Care* **36,** 3169–3176 (2013). Copyright and all rights reserved. Material from this publication has been used with the permission of the American Diabetes Association.

membrane (FIG. 2c)[21]. This pump drives sodium out of the cell in exchange for potassium. As the intracellular sodium concentration declines, sodium moves from the tubular lumen into the cell in a process that is coupled with glucose transport. As the intracellular glucose concentration increases, glucose moves passively from the cell to the interstitial space via the facilitative GLUT2 (in coordination with SGLT2) or GLUT1 (in coordination with SGLT1) transporters. The ratios of sodium to glucose cotransport are 1:1 and 2:1 for SGLT2 and SGLT1, respectively[21]. As sodium and glucose are cotransported in the proximal tubule, the enhanced glucose reabsorption in individuals with diabetes mellitus is associated with an increase in total body sodium and, not surprisingly, 60–70% of patients with T2DM eventually develop hypertension[34].

SGLT2 inhibition: principles and practice

Mechanism of action

Proof of concept that SGLT inhibition in the kidney could be an effective strategy for the treatment of diabetes was first provided in 1987 by Rossetti, DeFronzo and others[35,36]. In partially pancreatectomized diabetic rats, the combined SGLT2/SGLT1 inhibitor phlorizin normalized glycaemia by inducing glucosuria and correcting the deleterious effects of chronic hyperglycaemia on insulin sensitivity and insulin secretion[35–37]. Phlorizin is comprised of a glucose ring that binds to SGLT2 via *O*-glucoside to two phenol rings[21,32]. Given its low bioavailability and adverse gastrointestinal effects caused by inhibition of SGLT1, non-toxic agents that more specifically inhibit SGLT2 have been developed for the treatment of diabetes. Substitution of the *O*-link with a C-link renders the molecule resistant to β-glucosidase and enhances its bioavailability, whereas substitutions in the phenol rings increase selectivity for SGLT2 and prolong the molecule's half-life. Three SGLT2 inhibitors (dapagliflozin, canagliflozin and empagliflozin) have been approved by the FDA and European Medicines Agency in the USA and Europe, respectively, whereas tofogliflozin, luseogliflozin and ipragliflozin have been approved in Japan. Other SGLT2 inhibitors (remogliflozin, ertugliflozin and sotagliflozin) are under development.

In the most general sense, SGLT2 inhibitors can induce glucosuria by one of three mechanisms: by lowering Tm_G; by reducing the threshold for glucosuria; or by increasing splay. In diabetic rodents sergliflozin reduced Tm_G without altering threshold or splay[38]. In contrast, dapagliflozin[24] and empagliflozin (R.A. DeFronzo, unpublished work) reduced Tm_G, the renal threshold and splay in patients with diabetes, as assessed using the gold standard stepped hyperglycaemic clamp (FIG. 3). In patients with well controlled T2DM (that is, with HbA_{1c} levels ~6.5%) 2 weeks of dapagliflozin therapy decreased the Tm_G by 56%, from 420 mg per minute to 184 mg per minute[26]. Of note, the plasma glucose concentration at which the Tm_G was reached was ~8 mmol/l (~150 mg/dl). Nonetheless, when dapagliflozin is administered to normal glucose tolerant individuals with a fasting plasma glucose concentration of 4.4–5.0 mmol/l (80–90 mg/dl), marked glucosuria ensues. Further, many patients with T2DM have fasting plasma glucose <8 mmol/l (<150 mg/dl) yet respond well to SGLT2 inhibitors. These observations demonstrate that the reduction in Tm_G is insufficient to explain the glucosuria induced by SGLT2 inhibition. As the splay is also reduced by dapagliflozin[26], neither reduction in Tm_G nor change in splay can explain the glucosuric effect of SGLT2 inhibition. Rather, glucosuria is explained by a marked reduction in threshold from >11 mmol/l (200 mg/dl) to ~2.2 mmol/l (~40 mg/dl) (FIG. 3). Studies with canagliflozin suggest that this agent reduces the renal threshold for glucosuria to ~4.4 mmol/l (~80 mg/dl)[39,40]. However, this reduction is insufficient to explain the induction of glucosuria in individuals with normal fasting plasma glucose and is most likely explained by the imprecision of the methodology used to measure the threshold.

The contributions of SGLT1 and SGLT2

Although SGLT2 mediates the reabsorption of ~80–90% of filtered glucose (~160 g per 24 h in individuals with normal glucose tolerance), SGLT2 inhibitors only increase urinary glucose excretion by 70–80 g per day (less than 50% of the filtered glucose load)[26,41–43]. This paradox is explained by the anatomical location of SGLT2 and SGLT1 transporters and the unique characteristics of the transporters[29]. Given its proximal location in the S1 segment and large absorptive capacity, the SGLT2 transporter is the first to encounter filtered glucose and removes 80–90% of glucose from the filtrate. Consequently, the more distally located SGLT1 transporter in the S2/S3 segment is required to remove only the remaining 10–20%. Thus, SGLT1 operates well below its maximal transport capacity of ~80–100 g per day[29]. SGLT2 inhibition results in the delivery of a large amount of glucose to the SGLT1 transporter, which now can act at full reabsorptive capacity, explaining

Threshold for glucosuria
Represents the plasma glucose concentration at which glucose spillage into the urine is first observed.

Stepped hyperglycaemic clamp
A technique in which the plasma glucose concentration is raised by a fixed amount (for example, ~2.2 mmol/l) over a fixed time (for example, 30 minutes) to sequentially raise the plasma glucose concentration to 28–33 mmol/l (500–600 mg/dl). This technique enables the Tm_G and threshold for glucosuria to be calculated.

why less than 50% of filtered glucose appears in the urine[29]. Studies in *Sglt1*-knockout mice support this hypothesis[44,45]. Nondiabetic *Sglt1*-knockout mice have mild glucosuria, demonstrating that under nondiabetic conditions SGLT2 is capable of reabsorbing most of the filtered glucose; however, when treated with empagliflozin all of the filtered glucose appears in the urine. These results indicate that the difference between the amount of glucose that is filtered and the amount excreted during SGLT2 inhibition is reabsorbed by SGLT1 (REF. 46). In contrast to *Sglt1*-knockout mice, in which glucosuria is negligible, 30% of filtered glucose is excreted by mice lacking SGLT2 (REF. 45). When *Sglt1*-knockout mice are crossed with *Sglt2*-knockout mice, however, urinary glucose excretion increased threefold compared to levels in *Sglt2*-knockout mice[45]. These results provide conclusive evidence that the SGLT1 transporter can reabsorb ~30% of the filtered glucose and explain why SGLT2 inhibitors never produce the amount of glucosuria expected if SGLT2 were completely inhibited (that is, 80–90% of the filtered glucose load). *In vitro* studies using isolated perfused tubules provide further support of the quantitative importance of SGLT1 in renal tubular glucose reabsorption[47]. Glucose transport rates in isolated perfused rabbit proximal tubules were reported to be 93.4 ± 3.0 pmol per min per mm and 58.0 ± 8.4 pmol per min per mm for the early proximal (S1) and late proximal (S2/S3) convoluted tubule, respectively. From a quantitative standpoint, SGLT1 (which is present only in the S3 segment) can reabsorb 38% (58 pmol of the total 151.4 pmol) of the filtered glucose. Collectively, these results are consistent with our calculation in humans, whereby SGLT1 can reabsorb ~30–40% of glucose in the glomerular filtrate[29].

Combined SGLT2/SGLT1 inhibition

As glucose reabsorption by SGLT1 can blunt the glucosuric effect of SGLT2 inhibition, it follows that combined SGLT2/SGLT1 inhibitor therapy would markedly increase urinary glucose excretion and further reduce HbA_{1c} level. However, intestinal glucose absorption and GLP-1 secretion by L-cells in the distal small bowel and colon depend on SGLT1 (REF. 48), and inhibition of GLP-1 secretion could impair insulin secretion and result in worsening of glycaemic control. Studies in humans[48] and rodents[49] have, however, demonstrated that fermentation products of glucose (such as short chain fatty acids) are potent GLP-1 secretagogues and negate any inhibitory effect of SGLT1 inhibition on GLP-1 secretion. Further, gut SGLT1 activity can be inhibited by 40–50% without causing adverse gastrointestinal effects and this level of inhibition would be expected to reduce HbA_{1c} level by ~0.5%. In patients with T2DM[50] and in diabetic mice[49] the dual SGLT1/SGLT2 inhibitor sotagliflozin causes a modest increase in GLP-1 secretion following a glucose load. When combined with the dipeptidyl peptidase (DPP)-4 inhibitor, sitagliptin, plasma GLP-1 levels increased robustly in association with further improvements in glucose tolerance[49]. Although these results are encouraging, immunolocalization studies have identified SGLT1 protein in myocardial capillaries; thus the development of a combined SGLT1/SGLT2 inhibitor will require detailed studies to exclude any deleterious effect on the heart[51].

SGLT2 inhibition and glycaemic control

In nondiabetic individuals the three FDA-approved SGLT2 inhibitors — dapagliflozin, canagliflozin, empagliflozin — produce dose-related increases in urinary glucose excretion of ~60–80 g per day[39,41–43]. In a head-to-head study in individuals with normal glucose tolerance, canagliflozin (300 mg) increased urinary glucose excretion to a significantly greater extent than did dapagliflozin (10 mg; 51.4 g per day versus 40.8 g per day)[39]. The difference in urinary glucose excretion achieved with these two agents is explained by the longer half-life of canagliflozin, which results in higher plasma canagliflozin concentrations 16–24 h after drug administration. In individuals with T2DM all three SGLT2 inhibitors produce a dose-dependent increase in urinary glucose excretion and similar reductions in fasting and postprandial plasma glucose and HbA_{1c} levels[39,42,52,53], but no head-to-head comparative studies in diabetic patients are available.

Multiple publications have described the effect of canagliflozin, empagliflozin, and dapagliflozin on HbA_{1c} levels in drug-naive T2DM patients and as an add-on therapy in patients with poorly controlled T2DM (HbA_{1c} >7.0%) treated with metformin, sulfonylureas, pioglitazone, metformin plus sulfonylureas, metformin plus pioglitazone, and insulin compared to placebo or active comparators[32,53–55]. These studies demonstrate that in T2DM patients with HbA1c 7.8–8.2%, a reduction in HbA_{1c} of ~0.7–1.0% is achievable with SGLT2 inhibitor therapy. The reduction in HbA_{1c} and fasting plasma glucose levels are independent of the agent used. In short-term studies (<1 year duration), the decrements in HbA_{1c} achieved with SGLT2 inhibition are similar to those achieved by metformin, sulfonylureas, DPP-4 inhibitors, and pioglitazone. In long-term studies (>1 year duration) SGTL2 inhibitors cause a more durable reduction in HbA_{1c} than do sulfonylureas[56,57] and DPP-4 inhibitors[58,59] (FIG. 4). In 814 patients with poorly controlled T2DM on metformin, the reduction in HbA_{1c} with glipizide was similar to that achieved with dapagliflozin after 1 year. However, the HbA_{1c} reduction with glipizide was attenuated compared to dapagliflozin after 2 years and this difference persisted for up to 4 years[60]. Similar results were observed in a study that compared canagliflozin to glimepiride in 1,450 patients with poorly controlled T2DM who were treated with metformin[61].

Since the primary effect of SGLT2 inhibitors is to block glucose transport in the kidney, as long as kidney function is normal or only modestly reduced, these agents are equally effective in patients with newly diagnosed T2DM and in those with long standing T2DM (≥10 years duration) who are severely insulin resistant and insulinopenic[62]. In patients with insulin-treated T2DM (receiving ≥50 insulin units per day) 10 mg of dapagliflozin daily reduced HbA_{1c} by 0.78% after

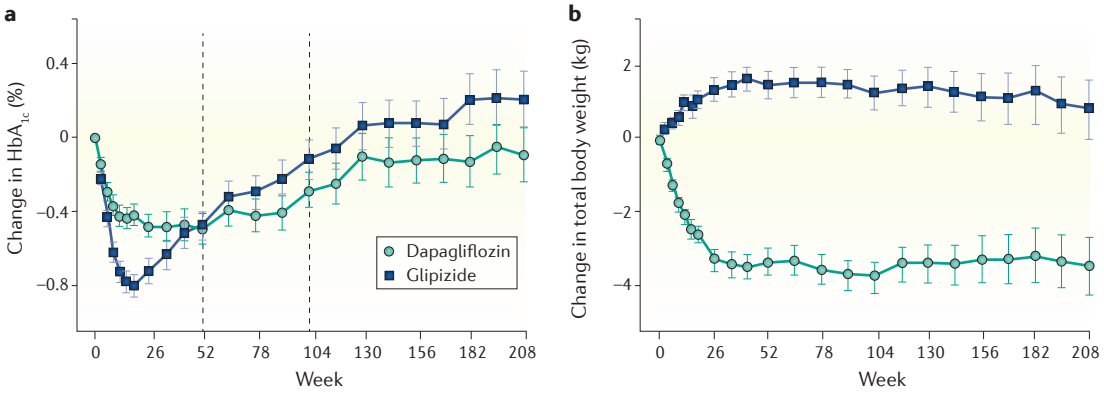

Figure 4 | **Effect of dapagliflozin on HbA_{1c} and body weight. a** | In patients with type 2 diabetes mellitus (T2DM) the initial decline (0–6 months) in HbA_{1c} is more rapid and greater with glipizide than with dapagliflozin. After 6 months, however, HbA_{1c} rises progressively in glipizide-treated patients and after 12 months the reduction in HbA_{1c} is significantly greater with dapagliflozin than with glipizide. **b** | Glipizide therapy in patients with T2DM is associated with significant weight gain. In contrast, dapagliflozin therapy was associated with a weight loss of 3–4 kg over 208 weeks. Of note, dapagliflozin-treated patients maintained their weight loss over the 4 year duration of the study. Modified with permission from Del Prato, S. *et al.* Long-term glycaemic response and tolerability of dapagliflozin versus a sulfonylurea as add-on therapy to metformin in patients with type 2 diabetes: 4-year data. *Diabetes Obes. Metab.* **17**, 581–590 (2015).

12 weeks despite a 50% reduction in the insulin dose[63]. Similar results were observed among patients with T2DM receiving ~70–80 insulin units per day and who received dapagliflozin[62], canagliflozin[64] and empagliflozin[65] for 48–52 weeks. Although not approved for patients with T1DM, all three SGLT inhibitors have been shown to reduce HbA_{1c} by 0.5–0.8% in these individuals without any change or reduction in insulin dose[66,67].

Patients with higher HbA_{1c} achieve greater reductions in HbA_{1c} than individuals with lower HbA_{1c}, regardless of the antidiabetic agent used[68]. Based on their renal mechanism of action, one would predict that, with progressively increasing plasma glucose concentration and HbA_{1c}, the efficacy of SGLT2 inhibitors not only would increase but exceed the glucose lowering effect of other antidiabetic agents, and clinical studies have confirmed this prediction[69,70]. In patients with poorly controlled T2DM and HbA_{1c} level <8.0% on metformin, the reduction in HbA_{1c} with dapagliflozin was slightly, but not significantly less than with saxagliptin (−0.45% versus −0.69%, respectively)[70]. By contrast, in T2DM patients with HbA_{1c} >9.0% (mean HbA_{1c} of 10.0%) a greater reduction in HbA_{1c} was achieved with dapagliflozin than with saxagliptin (−1.87% versus −1.32%). Thus, a 2.5% increment in baseline HbA_{1c} (7.5% to 10.0%) produced a greater than fourfold increase in the efficacy of the SGLT2 inhibitor but a lower than twofold increase in the efficacy of the DPP-4 inhibitor. These findings are explained by the greater amount of glucose filtered at higher plasma glucose concentrations, which is then targeted by SGLT2 inhibitor therapy. These observations have important clinical implications[71]. In drug naive patients with poorly controlled T2DM and an HbA_{1c} level of 10.0%, dapagliflozin reduced HbA_{1c} to 7.3% (a change of 2.7%). The addition of one extra antidiabetic agent would therefore be expected to reduce HbA_{1c} to target levels of 6.5–7.0%. Thus, in asymptomatic, diabetic patients with high HbA_{1c} (of 9–10%), such an approach might be an alternative to intensive insulin therapy.

First phase insulin secretion
The early response of insulin (within 0–10 minutes) to an acute intravenous injection of glucose.

Second phase insulin secretion
The late response of insulin (within 10–120 minutes) to a sustained rise in plasma glucose concentration brought about by a continuous intravenous infusion of glucose.

Mechanism of improved glycaemic control

SGLT2 inhibitors improve glycaemic control and reduce the plasma glucose level through two distinct mechanisms. First, by increasing the removal of plasma glucose by augmenting glucose excretion and second, by ameliorating glucotoxicity[37], which leads to improved insulin sensitivity in peripheral tissues[24] and enhanced β cell function[72,73]. Studies in diabetic animals treated with the nonspecific SGLT inhibitor phlorizin demonstrated that correction of hyperglycaemia improved both first phase insulin secretion and second phase insulin secretion[35], and increased muscle insulin sensitivity[37] by augmenting the translocation of the glucose transporter, GLUT4, from its intracellular domain to the cell membrane. These seminal studies from the late 1980s and early 1990s provided proof of concept that increasing renal glucose excretion could be an effective therapeutic intervention to improve glycaemic control in patients with T2DM. In individuals with poorly controlled T2DM, 2 weeks of dapagliflozin treatment reduced fasting plasma glucose levels by 1.9 mmol/l (35 mg/dl) and, similar to results in phlorizin-treated animals[35,36], improved insulin sensitivity by 25–30%[24] and β-cell function by approximately twofold[73] (FIG. 5). As the kidney is the primary site of action of SGLT2 inhibitors and since SGLT2 transporters have not been identified in muscle or β cells, the improvements in insulin sensitivity and insulin secretion with SGLT2 inhibition can be attributed to reversal of glucotoxicity[37].

Dapagliflozin treatment reduces glucose oxidation under both basal and insulin-stimulated conditions, increases fat oxidation[24,72,74–76], and modestly, but significantly increases plasma ketone concentrations. These changes in substrate oxidation arise as follows. The muscle and adipose tissue of individuals with T2DM is markedly resistant to insulin[14]. In order to meet the energy demands of the cell, the fasting plasma glucose concentration rises to a level that augments the mass

action effect of hyperglycaemia to enhance cell glucose uptake via the basal glucose transporters, GLUT2 and GLUT1 (REFS 14,77). Treatment with SGLT2 inhibitors leads to an acute increase in renal glucose excretion, leading to a precipitous fall in plasma glucose concentration. To meet their energy requirements, cells switch to lipid oxidation to generate energy in the form of ATP[24,73,75–77]. The end product of fat oxidation is acetyl CoA, which either can enter the Krebs cycle to be oxidized or be converted to ketone bodies. However, the Krebs cycle is impaired in patients with T2DM[14], and glucagon secretion by the α cell is stimulated by SGLT2 inhibition[22,24,73], leading to an increase in the glucagon:insulin ratio. These metabolic and hormonal changes favour the conversion of acetyl CoA to ketones (acetoacetate and β-hydroxybutyrate). In addition, insulin resistance in the adipocyte leads to enhanced lipolysis[78] and the resultant increase in plasma free fatty acid concentration leads to increased delivery of free fatty acids to the liver where they are converted to acetyl CoA via β oxidation and subsequently to ketones. As a corollary of the increase in lipid oxidation, we speculate that the concentration of toxic intracellular lipid metabolites (for example, fatty acyl CoAs, diacylglycerol and ceramides) is reduced, contributing to the reversal of lipotoxicity as evidenced by improvements in muscle insulin sensitivity and β-cell function[14,79]. Other studies have shown that treatment of patients with T2DM with empagliflozin induces similar effects, with reduced glucose oxidation, increased lipid oxidation, and elevated plasma ketone levels[72,76].

SGLT2 inhibition and the liver

Hepatic glucose production is the primary determinant of the fasting plasma glucose concentration[14]. Since SGLT2 inhibition consistently reduces fasting plasma glucose, SGLT2 inhibition might be expected to lead to reduced hepatic glucose output. On the contrary, when administrated to patients with T2DM following an overnight fast, dapagliflozin markedly increased hepatic glucose production and offset by ~50% the increase in urinary glucose excretion[24]. The increase in hepatic glucose production persisted for 2–4 weeks[24,72] and was associated with a small decline in fasting plasma insulin concentration and a marked increase in plasma glucagon concentration[24]. Although the increment in plasma glucagon was of sufficient magnitude to stimulate hepatic glucose production, the rise in glucagon did not occur until well after the increase in hepatic glucose production. We hypothesize that the rapid increase in hepatic glucose production following dapagliflozin administration can only be explained by neurogenic stimuli, resulting from activation of the renal nerves, which communicate directly with the liver via neural connections in the portal circulation or indirectly via neural connections from the kidney to the central nervous system and then to the liver. The latter mechanism also explains the inhibition of insulin and stimulation of glucagon secretion, although SGLT inhibitors are known to directly stimulate glucagon secretion by α cells[22].

As the increase in the basal rate of hepatic glucose production is associated with an increase in plasma glucagon and a decline in fasting plasma insulin concentration, combination therapy with a DPP-4 inhibitor should in theory blunt the rise in endogenous glucose production. Two studies[69,70], however, failed to demonstrate a significant beneficial effect of combined DPP-4 inhibitor/SGLT2 inhibitor therapy on glucose levels, despite blocking the rise in plasma glucagon with this approach[80]. Although hepatic glucose production was not specifically measured in these studies, the results have three possible explanations. First, DPP-4 inhibition was insufficient to block the SGLT2 inhibitor-induced rise in hepatic glucose production and a more potent inhibitor of hepatic glucose production, such as a GLP-1 receptor agonist, is required. Second, factors other than increased glucagon and decreased insulin — for example activation of the renal nerves — are responsible for the stimulation of hepatic glucose production. Third, the increase in endogenous glucose production might not emanate from the liver but rather from the kidney secondary to widening of the renal arteriovenous glucose concentration (due to inhibition of tubular glucose reabsorption), thus providing a stimulus for the kidney to increase glucose production.

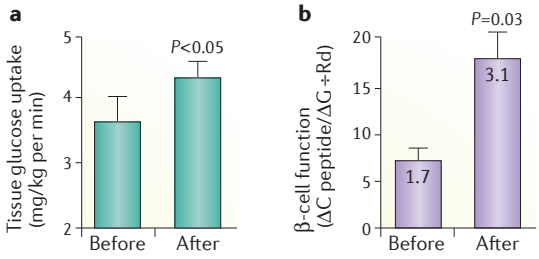

Figure 5 | **Effect of dapagliflozin on tissue sensitivity to insulin and β-cell function.** Treatment of patients with type 2 diabetes mellitus with dapagliflozin for 14 days led to a 25–30% improvement in muscle sensitivity to insulin (part **a**) and a nearly twofold increase in β-cell function (part **b**). The improvement in these two parameters resulted from the amelioration of glucotoxicity (that is, a reduction in plasma glucose concentration secondary to glucosuria) and contributed to improvements in overall glucose homeostasis. Part **a** modified with permission from Merovci, A. *et al. J. Clin. Invest.* **124**, 509–514 (2014). Part **b** modified with permission from Merovci, A. *et al. J. Clin. Endocrinol. Metab.* **100**, 1927–1932 (2015); permission conveyed through Copyright Clearance Center, Inc.

Effect on blood pressure and the kidney

As the reabsorption of glucose and sodium in the proximal tubule are coupled, SGLT2 inhibition is associated with mild negative salt and water balance and a durable decrease in extracellular fluid and plasma volumes[81]. The natriuretic effect of SGLT2 inhibition dissipates after 2–3 days and sodium and fluid balance is re-established, albeit with a ~7% reduction in plasma volume[81]. This modest reduction in extracellular fluid volume most likely accounts for the 5–6 mmHg decrease in systolic and 1–2 mmHg decrease in diastolic blood pressure observed within 1–2 weeks of

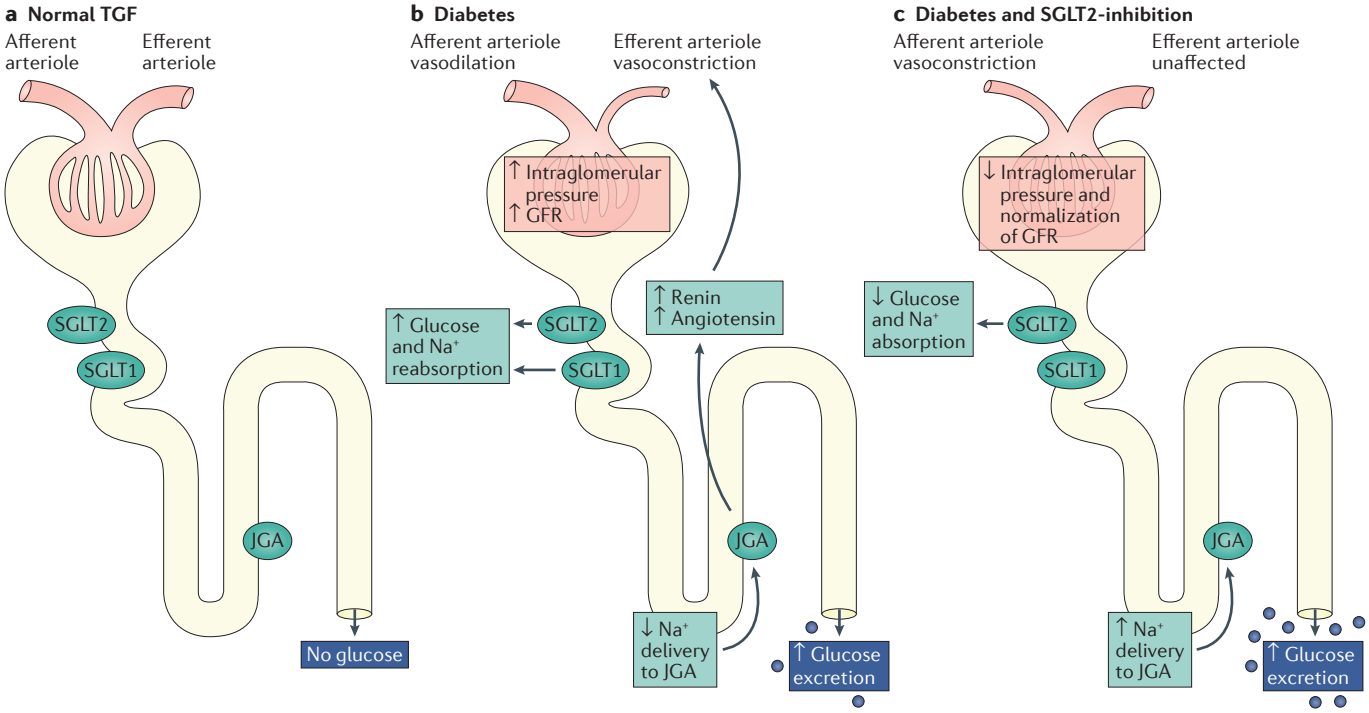

Figure 6 | **Effect of diabetes and SGLT2 inhibition on afferent and efferent arteriolar tone, glomerular filtration rate (GFR), and sodium (Na^+) excretion. a** | In normal glucose tolerant individuals the afferent renal arteriole arborizes into a diffuse capillary tuft that provides the surface area for glomerular filtration. The capillaries then coalesce to form the efferent arteriole. The glucose that is subsequently filtered is reabsorbed along with sodium by SGLT2 (~80–90%) and SGLT1 (~10–20%). As a result, no glucose reaches the juxtaglomerular apparatus (JGA) and normal tubuloglomerular feedback (TGF) is maintained. **b** | In hyperglycaemic individuals with poorly controlled diabetes the filtered glucose load is increased and glucose, along with Na^+, reabsorption is increased in the proximal tubule by both SGLT2 and SGLT1. This reabsorption reduces Na^+ delivery to the JGA, making the kidney seem under perfused. These effects lead to local release of renin and angiotensin, resulting in constriction of the adjacent efferent arteriole, and dilation of the afferent arteriole secondary to undefined neurohormonal factors. The net result of these intrarenal haemodynamic changes is an increase in intraglomerular pressure and GFR, which on a long-term basis can cause glomerular damage. **c** | Treatment with an SGLT2 inhibitor increases the delivery of glucose, along with Na^+, to the JGA, leading to afferent arteriole constriction, decreased intraglomerular pressure, and return of GFR to normal.

starting SGLT2 inhibitor therapy[53,54,82]. Over a period of 6–12 months, weight loss[53,54], alterations in the renin–angiotensin–aldosterone system[83], reduced plasma uric acid levels[84], decreased proteinuria[85], and other factors are also likely contribute to the sustained reduction in blood pressure[86].

The modest reduction in plasma volume following initiation of SGLT inhibitor therapy is associated with a small decline in glomerular filtration rate (GFR) of ~4–5 ml/min per 1.73m^2, which tends to return to baseline within 6–12 months of initiating therapy[87]. A growing body of evidence suggests that SGLT2 inhibition might afford renal protection and prevent the development of diabetic nephropathy. In hyperglycaemic animals, reabsorption of glucose and sodium is increased in the proximal tubule, resulting in decreased delivery of sodium to the juxtaglomerular apparatus[88–90], and increased local generation of renin and angiotensin, which leads to constriction of efferent arterioles and dilation of the afferent renal arterioles via changes in neurohormonal factors such as nitric oxide, adenosine and prostanoids (FIG. 6). The combination of efferent renal arteriolar vasoconstriction and afferent renal arteriolar vasodilation causes an increase in intraglomerular pressure, resulting in glomerular hyperfiltration and ultimately damage to the glomerulus. By blocking the transport of sodium along with that of glucose in the proximal tubule, SGLT2 inhibitors increase sodium delivery to the juxtaglomerular apparatus, which leads to afferent arteriolar vasoconstriction, decreased intraglomerular pressure, and return of GFR to normal (FIG. 6).

In diabetic mice, SGLT2 inhibition prevents progression of diabetic nephropathy, reduces proteinuria and normalizes glomerular mesangial area[91]. Consistent with these observations in rodents, treatment of patients with poorly controlled T1DM with empagliflozin for 8 weeks reversed their hyperfiltration and normalized GFR secondary to afferent renal arteriolar vasoconstriction[92]. As angiotensin-converting-enzyme (ACE) inhibitors reduce intraglomerular pressure and hyperfiltration by inducing vasodilation of efferent renal arterioles[93], combined therapy with SGLT inhibitors and ACE inhibitors might prove especially effective in preventing diabetic nephropathy. Consistent with this scenario, we have shown that normalization of plasma glucose levels with

intensive insulin therapy for 6 weeks in patients with T1DM reverses hyperfiltration and normalizes kidney size[94]. These intrarenal haemodynamic effects most likely explain the 30–40% reduction in microalbuminuria and macroalbuminuria observed following treatment of T2DM patients with empagliflozin and other SGLT2 inhibitors[95,96]. Although the glucosuria-mediated decreases in body weight and HbA_{1c} level are reduced in diabetic individuals with established diabetic nephropathy and reduced GFR (<60 ml/min per 1.73m^2), the blood pressure lowering and antiproteinuric effects of SGLT2 inhibitors remain intact[97]. In animal models of chronic diabetic kidney disease, increased intraglomerular pressure and glomerular hyperfiltration drive the progressive decline in GFR[88,90]. The ability of SGLT2 inhibitors to reduce proteinuria in patients with T1DM or T2DM is consistent with observations in rodents and suggests that they might be effective in slowing and/or preventing diabetic nephropathy. Further support for this hypothesis derives from the EMPA-REG OUTCOME study[11] in which 7,020 patients with T2DM at high risk of recurrent cardiovascular events were randomly assigned to receive empagliflozin or placebo. The primary composite end point of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke, was reduced by 14% (P=0.04). A 39% decrease (P<0.01) in the secondary composite renal end point (incident or worsening nephropathy, defined as a doubling of serum creatinine level accompanied by an estimated GFR <45 ml/min/1.73 m^2; time to dialysis, transplantation or renal death; or progression to macroalbuminuria) was also observed[13]. This later observation, if confirmed, has important implications for the prevention of diabetic nephropathy, which represents the most common cause of end-stage renal disease in Western countries. Since this renal protective effect was observed within 3.1 years and the reduction in $H1A_{1c}$ in the EMPA-REG OUTCOME study was modest (−0.28%), it is unlikely that improved glycaemic control explains the beneficial effect of empagliflozin on renal function. Rather, the nephroprotective effect is most likely is explained by changes in intrarenal haemodynamics.

Findings from ongoing studies will provide further insights into the renal and cardiovascular effects of SGLT2 inhibition. The CREDENCE trial[98] will recruit ~4,200 patients with T2DM and stage 2–3 chronic kidney disease and macroalbuminuria and is designed to examine whether canagliflozin can slow and/or prevent the progression of diabetic nephropathy. The end point of this study will be a composite of end-stage renal disease, doubling of serum creatinine, renal death (defined as initiation of dialysis or renal transplantation) or cardiovascular death. The CANVAS-R trial[99] will recruit ~5,700 patients with T2DM and a history of cardiovascular events or who are ≥50 years of age and at high risk of a cardiovascular event. The primary end point will be the development of microalbuminuria or macroalbuminuria in participants with baseline normoalbuminuria or the development of macroalbuminuria in participants with baseline microalbuminuria, accompanied by an increase in the urinary albumin to creatinine ratio of ≥30% from baseline. A key secondary end point in CANVAS-R will be a composite of cardiovascular outcomes, defined as cardiovascular death, nonfatal myocardial infarction or nonfatal stroke.

Effect on body weight

The increase in urinary glucose excretion with SGLT inhibitors amounts to a loss of ~60–80 g of glucose per day or 240–320 calories per day (1 g glucose equates to four calories). Over 14–15 days this loss of glucose leads to a caloric deficit of ~3,600 calories, which equates to a weight loss of or 450 g (1 lb). 6 months of SGLT2 inhibitor therapy typically results in a 2.5–3.0 kg reduction in body weight[32,53–55], which is associated with loss of visceral as well as subcutaneous adipose tissue[100,101]. However, despite continued glucosuria, this weight loss plateaus, which is likely due to an increase in caloric intake as has been documented in experimental animals[102] and humans[103]. The possibility that coadministration of a GLP-1 receptor agonist might attenuate the central hyperphagic drive that occurs with chronic SGLT2 inhibitor therapy and result in additive, even synergistic, weight loss is deserving of study in a prospective trial.

Effect on plasma lipids

In drug naive and metformin-treated patients with T2DM, SGLT2 inhibitors produce small 3–5% increases in LDL and ~5–8% increases in HDL cholesterol without any change in the ratio of HDL to LDL[104,105], and with a ~5% decrease in plasma triglyceride levels. The small rise in LDL cholesterol might relate to a reduction in LDL catabolism[106] and, as evidenced by the EMPA-REG OUTCOME results[11], does not seem to have any adverse effect on cardiovascular outcomes.

Effect on the central nervous system

As mentioned earlier, SGLT2 transporters are primarily localized to the kidney[21] but they are also found in human pancreatic α cells where they are involved in the regulation of glucagon secretion[22]. Small amounts of SGLT2 transporters are present in other tissues, including the cerebellum[21], but their function in these tissues is unknown.

Prevention of cardiovascular disease

Cardiovascular mortality is the primary cause of death in patients with T2DM[107]. Although tight glycaemic control can reduce and/or prevent microvascular complications, its effects on macrovascular complications are modest[1,7–9] and the beneficial effects of glucose lowering on the risk of myocardial infarction, stroke and cardiovascular mortality take up to 10 years to manifest[3,4]. As described above, the EMPA-REG OUTCOME study[11] showed that empagliflozin reduced the composite primary cardiovascular end point among patients with T2DM and a previous cardiovascular event or angiographically documented diffuse coronary artery disease, by 14% (HR 0.86, 95% CI 0.74–0.99, P=0.04). However, a striking disconnect was observed for the three outcome measures: nonfatal myocardial infarction (HR 0.87, P = 0.22); nonfatal

stroke (HR 1.24, P = 0.23); cardiovascular death (HR 0.62, P = 0.001; FIG. 7). Hospitalization for heart failure was reduced by 35% with empagliflozin treatment (HR 0.65, *P* = 0.002; FIG. 7). Statin[108] and antihypertensive[109] therapies also reduce the risk of these cardiovascular events, but their beneficial effects take ~1 year to manifest. In contrast, separation between the Kaplan-Meier curves for cardiovascular mortality and heart failure hospitalization between empagliflozin and placebo were observed within 3 months and progressively widened over a mean follow-up of 3.1 years. Several factors, including the failure of empagliflozin to reduce the risk of myocardial infarction and stroke, the absence of any improvement in unstable angina, and the rapidity of onset of reduction in cardiovascular mortality, suggest that the beneficial effect of empagliflozin on cardiovascular outcomes is not related to slowing of the atherosclerotic process and that other contributing factors are involved[86].

Potential mechanisms of cardiovascular protection. Potential mechanisms to explain the beneficial effects of empagliflozin on cardiovascular outcomes have been the subject of controversy[86,110–113] (FIG. 8). Improved glycaemic control is unlikely to explain the beneficial effect of empagliflozin on cardiovascular outcomes for multiple reasons. First, hyperglycaemia is a weak risk factor for cardiovascular disease[1]; second any beneficial effect of improved glycaemic control on cardiovascular events takes many years to manifest[3,4]; and last the reduction in HbA_{1c} with empagliflozin in the EMPA-REG OUTCOME study was modest — 0.45% at 90 weeks and 0.28% at 204 weeks[11], and similar to that achieved by with DPP-4 inhibition in three non-inferiority trials, which failed to demonstrate any cardiovascular benefit[114–116].

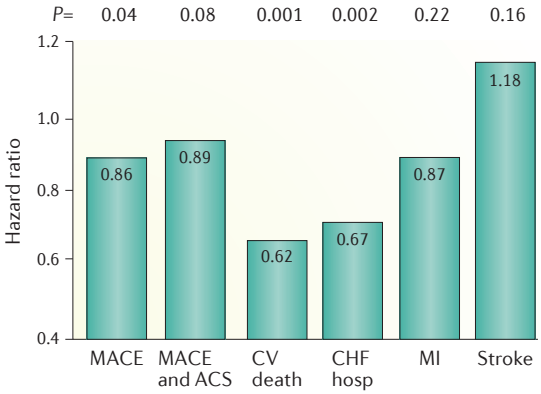

Figure 7 | **Effect of empagliflozin on cardiovascular events.** In the EMPA-REG OUTCOME study, 7,020 patients with type 2 diabetes mellitus at high cardiovascular risk were randomly assigned to receive empagliflozin or placebo with a mean follow up of 3.1 years. The primary end point (major adverse cardiovascular events (MACE)) was reduced by 15%. Of the three MACE end points, decreased cardiovascular (CV) mortality was primarily responsible for the cardiovascular benefit. Rates of myocardial infarction (MI) decreased modestly, whereas the risk of stroke increased modestly but neither change was significant. Empagliflozin had no beneficial effect on acute coronary syndrome (ACS), as indicated by the failure of the inclusion of ACS with MACE to alter the hazard ratio. Empagliflozin also had a marked effect on reducing hospitalization (hosp) for congestive heart failure (CHF). Data obtained from Zinman B *et al.* Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N. Engl. J. Med.* **373,** 2117–2128 (2015).

As mentioned earlier, SGLT2 inhibitors cause a shift in substrate oxidation from glucose to fat, which results in an increase in ketone production[76,112,113,117]. The amount of oxygen required to generate ATP from fat oxidation is greater than that required to generate the same amount from glucose and would be expected to cause myocardial ischaemia[118]. Therefore, a shift in fuel metabolism from glucose to fat is unlikely to explain the beneficial effect of empagliflozin on cardiovascular mortality and hospitalization for heart failure. The same is true for oxidation of ketones by the heart; however, the myocardium avidly extracts ketones and thus ketone body oxidation might lead to an improvement in myocardial efficiency[112,113,119]. Further studies are required to explore whether preferential ketone oxidation by myocardial cells provides an energetic advantage to the failing heart.

The reduction in blood pressure in the EMPA-REG OUTCOME study was ~5/2 mmHg and could explain, in part, the beneficial cardiovascular outcome. However, previous studies have shown that the beneficial effects of blood pressure lowering on cardiovascular risk take ~1 year to become apparent and have a greater effect on stroke than other cardiovascular events[109,120]. On the other hand, the decrease in brachial artery blood pressure might underestimate the decrease in central aortic pressure and does not provide information about arterial stiffness, both of which are major risk factors for cardiovascular disease[121,122]. SGLT2 inhibitors chronically reduce extracellular fluid and plasma volumes[81] as was evident in the EMPA-REG OUTCOME study with a 5.2% increase in haematocrit level at the study end[11]. This diuretic effect of SGLT2 inhibition would be expected to further decrease central aortic pressure and produce afterload reduction, leading to improved left ventricular (LV) function, reduced cardiac workload, and diminished myocardial oxygen demand. Preload reduction brought about by reduced plasma volume would be expected to act synergistically with afterload reduction to reduce cardiac events, especially in patients with diabetes and ischaemic heart disease, impaired LV function, or congestive heart failure. Unfortunately, no measurement of LV function or NT-proBNP, a marker of fluid volume, was performed. Although the diuretic hypothesis has been challenged[123], we think that the simultaneous reduction in afterload plus preload with SGLT2 inhibition is likely to have contributed to the reduction in cardiovascular mortality and heart failure in the EMPA-REG OUTCOME trial[6,124,125].

A reduction in intravascular volume can stimulate the renin–angiotensin–aldosterone system and aggravate cardiovascular disease by activating type 1 angiotensin (AT_1) receptors[83]. However, more than 80% of individuals in the EMPA-REG OUTCOME trial were treated

with ACE inhibitors or angiotensin-receptor blockers, which would be expected to favour activation of the AT_2 receptor and stimulation of the angiotensin 1–7 pathway, causing vasodilation, anti-proliferation of vascular smooth muscle cells, antiarrhythmic, anti-inflammatory and positive ionotropic effects[83]. Failure to observe any rise in heart rate despite a decrease in blood pressure and plasma volume suggests inhibition of cardiac sympathetic nerves, which could contribute to the observed reduction in cardiovascular mortality and hospitalization for heart failure. In the EMPA-REG OUTCOME trial, reduced cardiovascular mortality was observed in all diagnostic categories, including sudden death, worsening heart failure, acute myocardial infarction, stroke and cardiovascular death from other causes. The majority of deaths in the 'other' category resulted from arrhythmias, which are a major cause of sudden cardiac death. Decreasing stretch on the myocardium through simultaneous reduction in cardiovascular afterload and preload would be expected to decrease ventricular excitability and prevent arrhythmias, thereby contributing to the reduced risk of sudden cardiovascular death and cardiovascular death from 'other' causes.

SGLT2 inhibitors also promote the excretion of uric acid and in the EMPA-REG OUTCOME study, plasma uric acid concentrations declined by 42 µmol/l (0.7 mg/dl)[11]. Although previously considered to be an innocent bystander, mounting evidence suggests that elevated uric acid levels promote cardiovascular disease via a two-step process[84] starting with reversible hypertension, vascular inflammation and renal damage, which ultimately leads to atherosclerotic cardiovascular disease. Although this process cannot explain the early reduction in cardiovascular mortality within 3 months in the EMPA-REG OUTCOME study, it could contribute to the progressive reduction in cardiovascular disease observed between years 1–3. More importantly, the decrease in plasma uric acid concentration might have had an important role in the impressive slowing of diabetic nephropathy progression[84].

Obesity and insulin resistance are independent risk factors for atherosclerotic cardiovascular disease[6]. As mentioned previously, SGLT2 inhibitors induce weight loss; however, the magnitude of the weight loss and rapid reduction in cardiac mortality and heart failure in the EMPA-REG OUTCOME study suggest that weight reduction was not responsible for the cardiovascular benefit, which was observed early after initiation of empagliflozin therapy. It is possible, however, that weight loss could contribute to the progressive reduction in cardiovascular mortality and heart failure over years 1–3.

Other potential mechanisms that have been suggested to contribute to reduced cardiovascular mortality include increased glucagon secretion, a direct effect of the SGLT2 inhibitor on the myocardium, and a change in plasma electrolyte concentration and/or distribution. In humans, however, glucagon has no effect on LV function[126] and in animals glucagon receptor activation impairs cardiac contractility[127], making hyperglucagonaemia an unlikely candidate. SGLT2 transporters are not present in the myocardium,

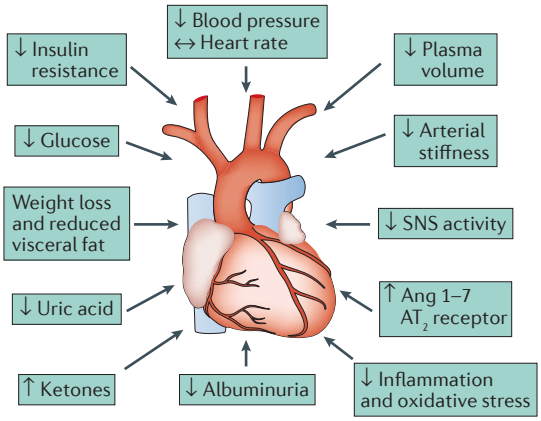

Figure 8 | **Potential mechanisms for the beneficial effect of empagliflozin on cardiovascular outcomes.** The EMPA-REG OUTCOME study was not designed to examine the mechanisms responsible for the cardiovascular benefit achieved with SGLT2 inhibition. However, haemodynamic factors, including reductions in blood pressure (after load), intravascular volume (preload), and aortic stiffness are likely to have contributed. The failure of heart rate to increase despite intravascular volume depletion suggests a potential role for reduced sympathetic nervous system activity. Of considerable interest is the ketone hypothesis, by which a switch from glucose to fat oxidation in the liver increases the plasma concentration of ketones that are preferentially taken up and oxidized as a fuel by the myocardium. Improved glycaemic control is unlikely to explain the cardioprotective effect of empagliflozin as the reduction in HbA_{1c} was very modest (–0.25%) and as any beneficial cardiovascular effect of improved glycaemic control takes up to 10 years to manifest, whereas the reductions in cardiovascular mortality and hospitalization for heart failure with SGLT2 inhibition were observed within 3 months. Weight loss and reduced visceral fat content could contribute to continued separation of the cardiovascular benefit of empagliflozin and placebo after 6–12 months but are unlikely to explain the cardiovascular benefit observed within the first 3 months. A number of other mechanisms (decreased plasma uric acid level, reduced inflammation and oxidative stress, activation of the angiotensin II receptor type 2 (AT_2), improved insulin sensitivity, and diminished albuminuria) have been suggested to explain the cardioprotective effective of empagliflozin but hard evidence to support any of these possibilities is lacking. Ang, angiotensin; SNS, sympathetic nervous system.

but SGLT1 transporters are. However, the inhibitory action of empagliflozin on SGLT1 is weak and the expected plasma-free empagliflozin levels in the EMPA-REG OUTCOME study are expected to be very low[86]. Further, if SGLT1 were inhibited by empagliflozin, the myocardial effect would be negative, not positive. No significant changes in plasma sodium, potassium or calcium concentrations were observed in the EMPA-REG OUTCOME study. Of note, SGLT2 inhibitors typically induce a negative sodium balance, which could cause a shift in sodium from the intracellular to the extracellular space. Such a shift in sodium distribution could lead to reduced interstitial myocardial fibrosis[128]; however, this effect takes

time[128] and is unlikely to explain the early reduction in cardiovascular mortality and hospitalizations for heart failure. Small 3–5% increases in serum phosphate and 7–9% increases in magnesium have been reported with SGLT2 inhibitors. However, we are not aware of any studies which suggest that such small changes could have any beneficial effects on cardiac function.

Are the EMPA-REG OUTCOME results generalizable or a class effect? All three approved SGLT2 inhibitors have similar effects on HbA_{1c}, blood pressure, body weight, and other metabolic and haemodynamic parameters[32,53–55]. Using a predictive model, dapagliflozin has been projected to reduce the risk of myocardial infarction, cardiovascular death, and all-cause death over a period of 20 years[129]; however, only the results of CANVAS-R and DECLARE will determine whether canagliflozin and dapagliflozin also reduce cardiovascular events. In the meantime, evidence-based medicine dictates that empagliflozin should be the SGLT2 inhibitor of choice for patients at high cardiovascular risk who are similar to those in the EMPA-REG OUTCOME study. No data are available to determine whether any of the three FDA-approved SGLT2 inhibitors will have a cardiovascular or renal protective effect in patients with T2DM without a high risk cardiovascular profile. Consequently, physicians should feel comfortable using any approved SGTL2 inhibitor in these patients, since these agents reduce HbA_{1c}, blood pressure, and body weight to a similar extent.

Safety of SGLT2 inhibitors

The major adverse effect of SGLT2 inhibition is genital mycotic infection which occurs in 7–8% of women and 1–2% of men, primarily uncircumcised males[32,53–55]. A small, statistically insignificant increase in urinary tract infections[32,53–55] and cases of urosepsis have also been reported[18]. An increase in adverse effects related to reduced intravascular volume has been documented, primarily in elderly diabetic patients and in patients treated with loop diuretics[32,53–55]. In these groups, the presence of orthostatic hypotension should be excluded before initiating therapy with an SGLT2 inhibitor. A small increase in urine volume and sodium excretion occurs during the initial 2–3 days of SGLT2 inhibitor therapy, and nocturia and more frequent urination are observed in 2–3% of individuals[54]. A small increase in serum urea nitrogen and decrease in estimated GFR can occur secondary to mild intravascular volume depletion, but these changes tend to return toward normal with continued therapy[32,53–55]. Further, the decrease in estimated GFR is most likely associated with decreased intraglomerular pressure, which would protect against renal damage, as discussed earlier. Hyperkalaemia is listed as an adverse effect of canagliflozin but has not been observed with other SGLT2 inhibitors. No consistent changes in serum sodium, chloride, bicarbonate, or calcium concentrations have been observed with SGLT2 inhibitor therapy. Hypoglycaemia is uncommon unless SGLT2 inhibitors are used with sulfonylureas or insulin[32,53–55]. Rare cases of ketoacidosis have been reported with SGLT2 inhibition[19,130] but most cases have been observed in situations known to be associated with diabetic ketoacidosis, such as patients with long-standing T2DM who are insulin deficient, partake in strenuous exercise, develop severe illness or undergo surgical procedures leading to release of adrenaline, those who ingest alcohol, and patients with poorly controlled T1DM (for whom SGLT2 inhibitors are not approved). An increase in bladder cancer has been reported with dapagliflozin but the number of cases is very small. In addition, most (seven of 10) patients had haematuria before entry into the study and most (nine of 10) patients developed bladder cancer within the first year of therapy[131]. Of note, SGLT2 is not expressed in bladder and, despite widespread use, a further signal for bladder cancer has not emerged[132]. In October 2015 the FDA issued a warning about bone fractures with canagliflozin[133]; however, no mechanistic link between fractures and SGLT2 inhibitor therapy has been identified[134], and the causality of this association is unclear. The FDA also has issued a warning about toe amputations with canagliflozin[135] but the number of cases is very small and a plausible causal explanation is not available.

As expected, the glucose lowering efficacy of SGLT2 inhibition begins to decrease when estimated GFR declines to <60 ml/min per 1.73 m^2 and SGLT2 inhibitors are not recommended in individuals with T2DM and estimated GFR <45 ml/min per 1.73 m^2. This reduction in efficacy is associated with a decrease in filtered glucose load (and thus less glucose to be inhibited) and tubular damage associated with advancing diabetic nephropathy. Of note, 26% of the study population in the EMPA-REG OUTCOME study had an estimated GFR <60 ml/min per 1.73 m^2 and experienced the same cardiovascular and renal benefits as those with higher GFR[13]. The efficacy of SGLT2 inhibition is easy to assess, and can be quickly determined by measuring either the decline in fasting plasma glucose or the fractional excretion of glucose following the first morning dose. A fractional excretion of glucose >30–40% is indicative of a good therapeutic response even in patients with estimated GFR is ≤45–60 ml/min per 1.73m^2.

Conclusions

As described in this Review, SGLT2 inhibitors effectively reduce HbA_{1c} directly by promoting glucosuria and indirectly by reducing glucotoxicity, leading to improved β-cell function and enhanced insulin sensitivity (FIG. 9). They also decrease body weight and blood pressure, reduce cardiovascular mortality and renal events, and have a good safety profile (FIG. 9). We therefore feel that SGLT2 inhibitors should be used early in the natural history of T2DM. Because of their unique mechanism of action on the kidney, they can be used as an initial therapy in drug-naive patients with T2DM or as an add-on therapy to any other antidiabetic agent, including insulin. Inhibition of proximal tubular sodium reabsorption by SGLT2 inhibitors leads to enhanced sodium delivery to the juxtaglomerular apparatus, a reduction in intraglomerular pressure,

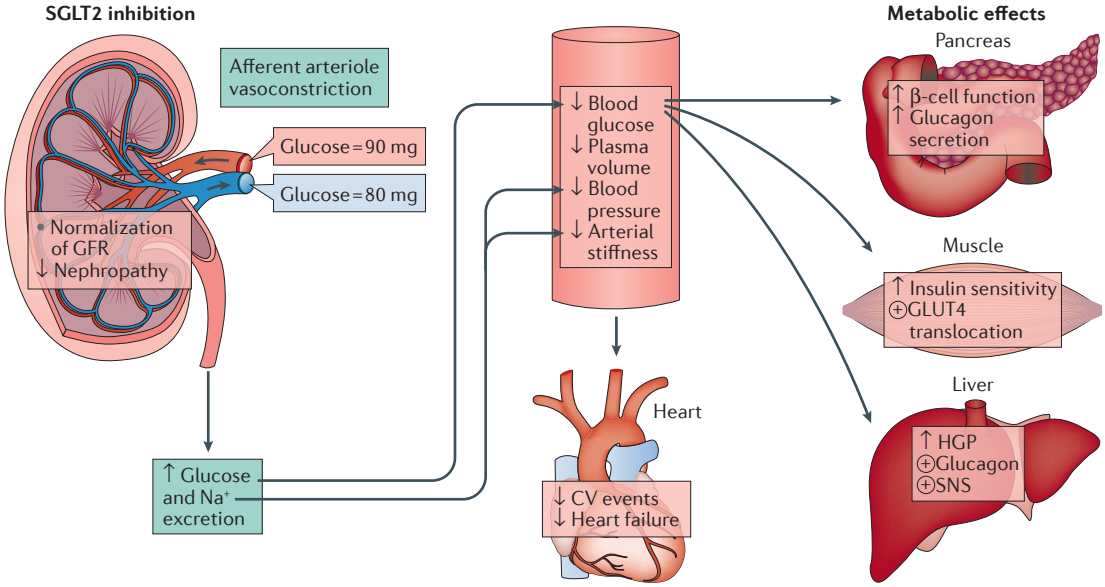

Figure 9 | **Beneficial effects of SGLT2 inhibition on glucose homeostasis and the cardiovascular and renal systems.** The primary effect of SGLT2 inhibitors is to promote the excretion of glucose and sodium (Na^+) by the kidney. In the kidney, this increased excretion results in afferent arteriole vasoconstriction, reduced intraglomerular pressure and normalization of the glomerular filtration rate (GFR) and provides protection against the development of diabetic nephropathy. Decreased renal glucose reabsorption results in a widening of the difference in arteriovenous glucose concentration, which can activate the renal nerves and transmit a neurogenic signal to the liver (and to the kidney) to augment hepatic (and renal) glucose production. The increase in urinary glucose excretion (60–80 g per day) reduces blood glucose concentration, ameliorating glucotoxicity and markedly augmenting β-cell function and muscle insulin sensitivity by promoting GLUT4 translocation to the cell membrane. Inhibition of SGLT2 in the α cell increases glucagon secretion which, in concert with activation of the renal nerves and sympathetic nervous system (SNS), stimulates hepatic glucose production. The decreases in blood pressure (after load reduction), plasma volume (preload reduction), and arterial stiffness lead to a reduction in ventricular volume, wall stress, and myocardial oxygen consumption leading to a decrease in cardiovascular (CV) events and heart failure. HGP, hepatic glucose production.

and normalization of hyperfiltration, suggesting these agents might be effective in preventing and/or slowing the progression of diabetic nephropathy (FIG. 9). Indeed, assessment of renal outcomes in the EMPA-REG OUTCOME study showed a significant reduction in the risk of worsening or incident nephropathy among patients with T2DM at high cardiovascular risk. The reduction in a composite of cardiovascular events, including cardiovascular mortality, and hospitalizations for heart failure with empagliflozin (FIG. 9) are impressive, and support a role for this agent as initial or add-on therapy in patients with T2DM at high cardiovascular risk. Although the beneficial cardiovascular and renal effects of the EMPA-REG OUTCOME study are likely to represent a class effect, this hypothesis remains to be established. Similarly, the mechanisms by which empagliflozin and other SGLT2 inhibitors protect cardiovascular and renal function remain to be elucidated. With respect to the prevention of diabetic nephropathy, studies employing combination therapy with SGLT2 inhibitors plus ACE inhibitors or angiotensin-receptor blockers will be of great interest.

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