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A comprehensive review of the pharmacodynamics of the SGLT2 inhibitor empagliflozin in animals and humans

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Abstract

Empagliflozin (formerly known as BI 10773) is a potent, competitive, and selective inhibitor of the sodium glucose transporter SGLT2, which mediates glucose reabsorption in the early proximal tubule and most of the glucose reabsorption by the kidney, overall. Accordingly, empagliflozin treatment increased urinary glucose excretion. This has been observed across multiple species including humans and was reported under euglycemic conditions, in obesity and, most importantly, in type 2 diabetic patients and multiple animal models of type 2 diabetes and of type 1 diabetes. This led to a reduction in blood glucose, smaller blood glucose excursions during oral glucose tolerance tests, and, upon chronic treatment, a reduction in HbA_{1c} in animal models and patients. In rodents, such effects were observed in early and late phases of experimental diabetes and were associated with preservation of pancreatic β-cell function. Combination studies in animals demonstrated that beneficial metabolic effects of empagliflozin may also manifest when added to other types of anti-hyperglycemic treatments including linagliptin and pioglitazone. While some anti-hyperglycemic drugs lead to weight gain, empagliflozin treatment was associated with reduced body weight in normoglycemic obese and non-obese animals despite an increased food intake, largely due to a loss of adipose tissue; on the other hand, empagliflozin preserved body weight in models of type 1 diabetes. Empagliflozin improved endothelial dysfunction in diabetic rats and arterial stiffness, reduced blood pressure in diabetic patients, and attenuated early signs of nephropathy in diabetic animal models. Taken together, the SGLT2 inhibitor empagliflozin improves glucose metabolism by enhancing urinary glucose excretion; upon chronic administration, at least in animal models, the reductions in blood glucose levels are associated with beneficial effects on cardiovascular and renal complications of diabetes.

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Keywords

Type 2 diabetesmellitus; Obesity; Empagliflozin; diabetic nephropathy; endothelial dysfunction; blood pressure

Introduction

Type 2 diabetes mellitus is a disease with a globally growing prevalence (Scully 2012), attributable at least in part to a growing prevalence of obesity (Scully 2014). Diabetes has a severe impact on the afflicted patients, leading to disabling morbidity and mortality, at least partly via micro- and macrovascular complications; accordingly, diabetes places a huge economic burden on society (Adler et al. 2003). Most classic anti-hyperglycemic medications including insulin, insulin secretagogues (sulfonylureas, meglitinides), and insulin sensitizers (thiazolidinediones) are often associated with further weight gain in type 2 diabetes patients (Purnell and Weyer 2003). This feeds the vicious cycle of higher body weight placing an increasing strain on pancreatic β-cells, thereby leading to an even greater treatment requirement. Biguanides and α-glucosidase inhibitors produce less if any weight gain but in many patients, at least as monotherapies, produce insufficient control of glucose homeostasis. While microvascular complications such as diabetic nephropathy, retinopathy, and neuropathy are believed to be directly related to glycemic control, macrovascular complications may be inadequately addressed by most existing anti-hyperglycemic therapeutics.

Against this background, the idea has evolved that promotion of urinary glucose excretion may be a promising approach to reduce glucose load (List and Whaley 2011; Abdul-Ghani et al. 2011; Bailey 2011). Renal glucose reabsorption largely occurs in the proximal tubules. Glucose is transported by sodium glucose transporters (SGLT) across the luminal membrane, with SGLT2 being the main pathway for such reabsorption. This concept on renal glucose handling was initially developed based on the characteristics of isolated proximal tubules and their transport vesicles, the properties of the proteins encoded by cloned SGLT2 and SGLT1, and the phenotypes of humans with mutations in the corresponding genes while the potential of spilling glucose into the urine as a blood glucose lowering approach emerged from the use of rather non-selective SGLT inhibitors such as phlorizin (Wright et al. 2011). These concepts were more recently substantiated using mice carrying a non-sense mutation of the SGLT2-encoding gene Slc5a2 (Ly et al. 2011) and by direct in vivo renal micropuncture in mice genetically engineered to lack SGLT2 (Vallon et al. 2011). The studies in gene-targeted mice have confirmed that SGLT2 is considerably more important for renal glucose reabsorption than SGLT1 (Vallon et al. 2011; Gorboulev et al. 2012; Powell et al. 2013; Vallon et al. 2013; Rieg et al. 2014). The renal phenotype of SGLT2 knockout mice is consistent with that of individuals with familial renal glucosuria who carry specific mutations in the *SLC5a2* gene (Vallon et al. 2011). Importantly and even though the number of affected individuals is small, humans with mutations in SGLT2 do not show signs of general renal tubular dysfunction or other pathological changes and seem to have normal life expectancy (Santer and Calado 2010; Vallon and Sharma 2010; Wright et al. 2011). Therefore, scientists in academia (Vallon and Sharma 2010; Cangoz et al. 2013;

Tahrani et al. 2013; Ferrannini and Solini 2014), industry (Isaji 2011), and regulatory bodies (Mayer 2012) came to the conclusion that inhibition of SGLT2 may represent a pathophysiologically sound concept for the treatment of type 2 diabetes.

Empagliflozin (formerly known as BI 10773, Fig. 1) is a specific SGLT2 inhibitor belonging chemically to the C-glucoside family (dapagliflozin, canagliflozin, empagliflozin, ipragliflozin, and tofogliflozin). This manuscript will focus on the pharmacodynamics of empagliflozin which recently has been approved for clinical use in type 2 diabetes in several countries. Empagliflozin effects will be compared with those of SGLT2 inhibition by genetic approaches. The pharmacokinetics of empagliflozin have comprehensively been reviewed elsewhere (Scheen 2014).

Effects at the molecular and cellular level

The affinity of tritiated empagliflozin for its molecular target was directly determined by kinetic radioligand binding studies using membranes from a cell line transfected with the human SGLT2 (Grempler et al. 2012b). This yielded a half-life of empagliflozin binding of 59 min and an affinity estimate of 57 nM. A high physiological glucose concentration lowered the association rate of empagliflozin and hence the apparent empagliflozin affinity calculated from it, demonstrating the competitive nature of its binding to the transporter.

The inhibition of monosaccharide transporters by empagliflozin and related compounds was compared in cell lines stably transfected with human SGLT1, SGLT2, SGLT4, SGLT5, and SGLT6 using appropriate radiolabelled substrates (α-methyl glucopyranoside, mannose, and myo-inositol) (Grempler et al. 2012b). In such experiments, empagliflozin showed high potency at SGLT2 (IC_{50} 3.1 nM) and much lower potency at the other transporters (IC_{50} 1100–11,000 nM), resulting in a high degree of selectivity. In comparison to other SGLT inhibitors, empagliflozin had a very high degree of selectivity for SGLT2 as compared to SGLT1 (Fig. 2). The high selectivity of empagliflozin and several other SGLT2 inhibitors for human SGLT2 vs. SGLT5 was confirmed in another study by the same group of investigators (Grempler et al. 2012a); in those experiments, the inhibitory potency of empagliflozin at SGLT5 was similarly low irrespective of the SGLT5 substrate being used, i.e., α-methyl-D-glucose, mannose, or fructose.

In similar experiments, empagliflozin was more potent for the rat SGLT1 (but not murine) as compared to the human SGLT1, making it less selective for SGLT2 in rats than in humans (Grempler et al. 2012b), an observation also made for other SGLT2 inhibitors such as dapagliflozin (Han et al. 2008) and apparently related to species differences in critical amino acids in the transporter protein. As described for human SGLT2 (see above), the interaction of empagliflozin and glucose at the rat SGLT1 was competitive (Grempler et al. 2012b). In contrast, empagliflozin did not inhibit the facilitative glucose transporter GLUT1 in concentrations up to 10 μM (Grempler et al. 2012b). While not specifically tested for empagliflozin, other SGLT2 inhibitors have been reported to be ineffective on GLUT2 (Meng et al. 2008; Kurosaki and Ogasawara 2013).

In vivo effects

Studies of glucose homeostasis in euglycemic animals and healthy humans

Several studies have explored effects of SGLT2 inhibition in non-diabetic animals and humans. Mouse studies with genetic SGLT inactivation have used the Sweet Pee mouse, which carries a non-sense mutation of the SGLT2-encoding gene Slc5a2 (Ly et al. 2011) or knockout approaches (Jurczak et al. 2011; Vallon et al. 2011; Vallon et al. 2013). The Sweet Pee mouse exhibited improved glucose tolerance, higher urinary excretion of calcium and magnesium, and growth retardation (Ly et al. 2011). The model showed distal osmotic diuresis without enhanced natriuresis; markers of acute tubular injury were not increased. In comparison with wild-type animals, SGLT2 knockout mice exhibited a phenotype with glucosuria, polyuria, and increased food and fluid intake; this was associated with normal plasma glucose concentrations, glomerular filtration rate (GFR), and urinary excretion of other proximal tubular substrates including amino acids (Vallon et al. 2011; Vallon et al. 2013). While there was no evidence of body weight change or major volume depletion, a minor increase in plasma renin and a decrease in plasma aldosterone levels was observed; the latter may serve to conserve potassium when an osmotic diuresis facilitates renal potassium loss (Vallon et al. 2011). In whole-kidney clearance studies, fractional glucose reabsorption was significantly reduced in SGLT2 knockout mice, with the extent of reduction depending on the amount of filtered glucose. Free-flow micropuncture experiments demonstrated that glucose reabsorption in early proximal tubules was absent in SGLT2 knockout mice, while it accounted for almost 80 % of fractional reabsorption in wild-type animals. In late proximal collections, glucose reabsorption became evident in the knockout mice, consistent with partial compensation by SGLT1. These studies established that SGLT2 protein is expressed in the luminal membrane of the early proximal tubule and mediates all glucose reabsorption in the early proximal tubule and most of the overall renal glucose reabsorption (Vallon et al. 2011), consistent with the glucosuric phenotype of individuals carrying inactivating *SLC5a2* mutations.

Additional studies were performed with pharmacological SGLT2 inhibition in euglycemic mice, often the control group for diabetic mouse models used in the same study. In C57Bl/6 mice, acute dosing of empagliflozin significantly reduced glucose excursion during an oral glucose tolerance test (OGTT) to a similar extent as the dipeptidylpeptidase 4 inhibitor linagliptin, and the combination of the two agents had a greater effect than either monotherapy (Thomas et al. 2011). In wild-type mice, acute oral dosing of empagliflozin dose-dependently increased urinary glucose excretion with an ED_{50} of 1.2 mg/kg (Fig. 3a).

In normoglycemic diabetic mutant (db/m) mice, a 7-day treatment with empagliflozin transiently lowered blood glucose and caused a maintained increase in 24-h urinary glucose excretion, urine volume, and sodium excretion; this was accompanied by a reduction of body weight gain and increase of water intake (Lin et al. 2014). At study end, when glucose levels had returned to baseline, insulin levels were not different.

A 3-week administration of empagliflozin increased urinary glucose concentration and glucose/creatinine ratios in wild-type mice; maximum effects occurred after 2 days of treatment and were maintained throughout the remaining treatment period (Rieg et al. 2014).

This was mirrored by an increase in food and water intake, resulting in an only transient lowering of blood glucose. Subsequent renal inulin clearance studies revealed that fractional glucose reabsorption in these chronically treated mice was 64 % (Fig. 3b); this was seen at a free plasma empagliflozin concentration of 1–2 nM (Fig. 3c), i.e., in the range of drug IC_{50} at its molecular target (see Fig. 2). To determine ceiling effects, a high dose of empagliflozin was acutely given to such mice, increasing free plasma concentrations to 20–22 nM (Fig. 3c); this lowered fractional glucose reabsorption to 44 % (Fig. 3b), which was similar to the values (36 %) reported in SGLT2 knockout mice by the same investigators (Vallon et al. 2011). In a 15-week treatment study in non-diabetic mice, empagliflozin increased renal membrane SGLT2 protein expression by 47 % and reduced the expression of SGLT1. The effect of empagliflozin on renal SGLT1 expression was consistent with findings in SGLT2 knockout mice (Vallon et al. 2013). Empagliflozin induced sustained increases in urinary glucose/creatinine ratios and modestly lowered blood glucose levels without affecting plasma insulin concentrations.

Basal urinary glucose excretion was modestly enhanced in SGLT1 knockout mice representing ~3 % of filtered glucose (Gorboulev et al. 2012); in such mice, empagliflozin dose-dependently increased glucose excretion, with greater potency (ED_{50} of 0.4 mg/kg) and to a greater extent than in wild-type mice (Fig. 3a) (Rieg et al. 2014). All effects of a 3-week empagliflozin treatment described above were enhanced in SGLT1 knockout mice, leading to a temporary reduction in blood glucose and, despite a further enhanced food and fluid intake, to a weight loss of about 3 %. Subsequent renal clearance studies demonstrated that an increase in SGLT1-mediated glucose uptake explained why full inhibition of SGLT2 by empagliflozin only excreted 50–60 % of the filtered glucose in euglycemic mice (Fig. 3b, c). In the absence of SGLT1, full inhibition of SGLT2 (using high-dose empagliflozin (Fig. 3b) or SGLT2 knockout) completely abolished net renal glucose reabsorption (Rieg et al. 2014).

Studies in euglycemic rats were largely limited to those with diet-induced obesity. In such animals, a 4-week empagliflozin treatment (10 mg/kg/day) increased urinary glucose excretion and, to a lesser extent, urine volume; daily food intake was not affected and only minor increases in fluid intake were observed (Grempler et al. 2010). A similarly designed follow-up study from the same group confirmed these effects in response to empagliflozin and also demonstrated a lowered plasma insulin in this model (Grempler et al. 2011).

Data on empagliflozin effects on glucose homeostasis in euglycemic humans are available from phase I studies in Caucasian and Japanese subjects. In Caucasian subjects, single oral dosing of empagliflozin at 0.5–800 mg dose-dependently increased urinary glucose, with a 40 % inhibition of reabsorption in doses up to 10 mg and approximately 40–60 % with higher doses, reaching a plateau at a dose of about 100 mg (Seman et al. 2013). The dose-dependent increase in urinary glucose excretion was confirmed with single oral doses of 1–100 mg empagliflozin in Japanese subjects; plasma glucose was not affected (Sarashina et al. 2013). In summary and consistent with consequences of genetic loss-of-function SGLT2 in mice and humans, application of empagliflozin causes urinary glucose excretion in non-diabetic mice, rats, and humans.

Studies of glucose homeostasis in type 2 diabetes

Acute studies of glucose homeostasis in type 2 diabetes models and patients

—Acute effects of empagliflozin on glucose homeostasis have been tested in db/db mice carrying an inactivating mutation of the leptin receptor, Zucker diabetic fatty rats, and type 2 diabetes patients. In db/db mice, a single dose of empagliflozin increased urinary glucose excretion to 17.7 nmol glucose/kg body weight (Eickelmann et al. 2009). This was accompanied by a lowering of blood glucose by empagliflozin for at least 7 h. Moreover, in an OGTT, 1 mg/kg empagliflozin reduced the glucose area under the curve (AUC) by 35 %. An empagliflozin-induced lowering of blood glucose along with an increase of 24-h urinary glucose excretion, urine volume, and sodium excretion in db/db mice was confirmed by other investigators (Lin et al. 2014).

In Zucker diabetic fatty rats, pancreatic β-cell function declines over time leading to decreased insulin and increased glucose and HbA_{1c} levels, i.e., a model mimicking the course of type 2 diabetes in patients. Acute oral administration of empagliflozin dose-dependently increased urinary glucose excretion in 10-week-old and lowered blood glucose in 13-week-old Zucker rats (Thomas et al. 2012). Peak glucose lowering was observed about 3 h after administration and the maximum effect on blood glucose was −11.4 mM at the 3 mg/kg dose as compared to −2.5 mM in the vehicle group. The ED_{50} of empagliflozin for glucose lowering estimated from these experiments was 0.6 mg/kg. The glucose lowering effect was maintained for at least another 4 h (end of experiment). This corresponded to pharmacokinetic findings in Zucker rats showing a t_{max} of about 2 h and a $t_{1/2}$ of 1.5 h; of note, an earlier t_{max} but a much longer $t_{1/2}$ was seen in dogs (1 and 6.3 h, respectively) (Grempler et al. 2012b). Empagliflozin also improved glucose excursions during an OGTT in this model (Thomas et al. 2011).

To determine how different levels of deterioration in pancreatic β-cell function affect responses to anti-hyperglycemic drugs, OGTTs were performed in 7–27-week-old Zucker rats after single doses of empagliflozin (10 mg/kg orally), the biguanide metformin (300 mg/kg orally), the GLP-1 agonist liraglutide (150 μg/kg subcutaneously), the sulphonylurea glipizide (10 mg/kg orally), or vehicle (Mayoux et al. 2013). In 9-week-old Zucker rats, i.e., just at the beginning of diabetes, liraglutide reduced the glucose AUC by 43 % relative to vehicle as compared to 30–33 % by the other three drugs. In 15-week-old rats, glipizide was no longer effective, the effect of liraglutide was diminished to 29 %, whereas those of metformin and empagliflozin were enhanced to 41 and 50 %, respectively. In 27-week-old animals, glipizide again was ineffective, the effect of liraglutide had weakened further to 18 %, whereas those of metformin and empagliflozin remained effective with reductions in glucose AUC of 42 and 45 %, respectively. These findings suggested that with worsening of type 2 diabetes, drugs that depend on pancreatic β-cell function, such as sulfonylurea derivatives and liraglutide, loose efficacy, whereas those acting independent of β-cell function, such as metformin or empagliflozin, maintain their effects on glucose homeostasis.

In line with the acute effects of empagliflozin on urinary glucose excretion in mice and rats, increases in urinary glucose excretion were also consistently observed in type 2 diabetes

patients receiving 1–100 mg empagliflozin starting from the first day of treatment in Caucasian (Heise et al. 2013a, b) and Japanese patients (Kanada et al. 2013).

Chronic studies of glucose homeostasis in type 2 diabetes models and patients—During a 7-day empagliflozin treatment, db/db mice exhibited a sustained lowering of blood glucose; however, the increase of 24-h urinary glucose excretion, urine volume, and sodium excretion seen on day 1 was not observed from day 2 onward up to day 7 (Lin et al. 2014). In a second set of experiments within this study, the lowering of plasma glucose was maintained for 10 weeks, and glucose excursions during an OGTT were reduced to levels similar to those in euglycemic db/m mice (Lin et al. 2014). At study end, serum insulin levels were close to those of euglycemic mice in vehicle-treated and markedly elevated in empagliflozin-treated db/db mice.

In db/db mice, an 8-week treatment with empagliflozin dose-dependently lowered HbA_{1c} by up to 35 % and improved glucose levels during an OGTT (Kern et al. 2012). In euglycemic-hyperinsulinemic clamp experiments in such mice, empagliflozin dose-dependently improved insulin sensitivity and reduced hepatic glucose production. Moreover, a reduction in hepatic triglyceride content, serum triglycerides, and circulating free fatty acids was observed. In a follow-up study, these investigators compared effects of an 8-week treatment with empagliflozin (10 mg/kg/day), linagliptin (3 mg/kg/day), or their combination with those of vehicle using euglycemic, hyperinsulinemic clamp experiments in female db/db mice (Klein et al. 2014). Compared to vehicle, all three treatments suppressed insulin-mediated hepatic glucose production, enhanced glucose uptake in liver, improved glucose disposal rate, and lowered hepatic triglyceride content; glucose uptake into muscle and adipose tissue was not affected by any treatment. In another study with db/db mice, empagliflozin treatment for 4, 8, and 12 weeks lowered HbA_{1c}, blood glucose, and plasma insulin (Jelsing et al. 2012). Glucose levels during an OGTT after 1 or 10 weeks of treatment were also improved. In another diabetes model (BTBR leptin-deficient (ob/ob) mice), a 21-day empagliflozin treatment increased glucosuria and lowered blood glucose (Gembardt et al. 2014).

In 12-week-old Zucker diabetic fatty rats, blood glucose and HbA_{1c} increased from baseline to study end during a 5-week vehicle treatment; empagliflozin treatment dose-dependently lowered blood glucose and HbA_{1c} yielding HbA_{1c} values even below baseline levels with the higher empagliflozin doses (Fig. 4). The glucose AUC during an OGTT was not significantly affected on day 2 but reduced on day 37 by empagliflozin, indicating an enhanced glucose tolerance with improved insulin sensitivity. Four days after the last dose, animals were subjected to a hyperinsulinemic-euglycemic clamp test. In this test, the glucose infusion rate was significantly higher with empagliflozin than vehicle (43.5 vs. 9.3 μmol/min/kg), supporting an improved insulin sensitivity. Sustained beneficial effects of empagliflozin on blood glucose, HbA_{1c}, and OGTT in Zucker diabetic fatty rats were confirmed in other studies with 4–8 weeks of treatment (Hansen et al. 2014).

In the latter study, effects of once daily empagliflozin were compared to those of the sulphonylurea glibenclamide and the long-acting GLP-1 analog liraglutide (Hansen et al. 2014). While empagliflozin and liraglutide markedly improved fed levels of glucose, insulin,

and HbA_{1c}, glibenclamide was ineffective at the dose being used. Similarly, glucose levels during an OGTT under semi-fasted conditions after 4 and 8 weeks of treatment were improved by empagliflozin and liraglutide but not by glibenclamide. While effects of empagliflozin were very similar at both time points, those of liraglutide tended to weaken over time, as had been observed in the mouse studies (see above). In another study, 7-week-old Zucker diabetic fatty rats were treated with empagliflozin (10 mg/kg/day) or vehicle for 6 or 14 weeks (Pepin et al. 2014). Empagliflozin abolished the increase in fasting plasma glucose (FPG) and HbA_{1c} levels observed in vehicle-treated rats over the 14-week study period. Concomitantly, the decline in plasma insulin was attenuated by empagliflozin. During hyperglycemic clamps at study end, plasma insulin levels, C-peptide levels, and maximal insulin release in response to arginine were higher in empagliflozin- vs. vehicle-treated rats, indicating that empagliflozin treatment had delayed the onset of hyperglycemia in the aging Zucker rats by preserving insulin secretory capacity.

Lowering of fasting and post-prandial blood glucose was also reported upon treatment with empagliflozin (10 mg/kg/day) for 18 weeks in Cohen Rosenthal diabetic hypertensive rats (Younis et al. 2014b). Within that study, empagliflozin treatment improved blood glucose excursion during intraperitoneal glucose tolerance tests performed after 80 and 120 days of treatment (Younis et al. 2014a). Similar effects were observed with empagliflozin (20 mg/kg/day) when applied for 8 or 12 weeks in hypertensive rat models of type 1 and type 2 diabetes; these included Dahl salt-sensitive rats fed a high-salt diet and that received an streptozotocin (STZ) injection and Goto-Kakizaki rats implanted with a long-acting deoxycorticosterone acetate pellet receiving 1 % NaCl in drinking water, respectively (Murphy et al. 2014).

The above alterations of glucose metabolism apparently involve not only a functional improvement but also morphological changes of the pancreas. Thus, pancreatic β-cell mass at study end was increased by empagliflozin as compared to vehicle in db/db mice, whereas pancreatic non-β-cell mass was not affected (Jelsing et al. 2012). Compared to baseline values, vehicle- and glibenclamide-treated Zucker rats exhibited a reduced β-cell mass at 4 and 8 weeks; liraglutide prevented this β-cell loss at the 4- but not the 8-week time point, whereas empagliflozin prevented it at both time points (Hansen et al. 2014).

While a detailed analysis of the many clinical studies with empagliflozin is not within the scope of this manuscript, it should be noted that in line with the above animal studies, increased urinary glucose excretion has consistently been observed in type 2 diabetes patients upon longer-term (up to 4 weeks) treatment with empagliflozin. The glucosuric effect was noticed immediately upon start of treatment and maintained without evidence of desensitization throughout the course of the study (Heise et al. 2013a, b; Kanada et al. 2013).

The above data demonstrate that chronic empagliflozin treatment lowers plasma glucose and HbA_{1c}. Similar to studies with the experimental SGLT2 inhibitor AVE2268 in db/db and diabetic Tallyho/JngJ mice (Neschen et al. 2015) and with dapagliflozin in insulin-resistant mice (Bonner et al. 2014) and in diabetic patients (Merovici et al. 2014), a single dose or a 4-week treatment with empagliflozin increased endogenous glucose production

accompanied by elevated plasma glucagon concentrations in patients with type 2 diabetes; this may occur secondary to an increase in glucagon synthesis and release as shown in isolated human pancreatic α-cells and in in vivo in insulin-resistant mice (Bonner et al. 2014).

Combination treatment studies in type 2 diabetes—Some studies have explored effects of combinations of empagliflozin with other anti-hyperglycemic agents in mice and rats. In 8-week-old female db/db mice, treatment with empagliflozin (10 mg/kg), linagliptin (3 mg/kg), or their combination for 8 weeks improved glucose disposal, assessed by euglycemic-hyperinsulinemic clamps, by 5.9, 3.4, and 7.8 mg/kg/min, respectively, as compared to 1.9 mg/kg/min upon vehicle treatment; combination treatment was also superior to both monotherapies in reducing hepatic lipid content (Klein et al. 2014). While empagliflozin had increased endogenous glucose production in patients with type 2 diabetes, empagliflozin alone or in combination with linagliptin enhanced insulin-mediated suppression of hepatic glucose production in db/db mice. Compared with vehicle, empagliflozin, linagliptin, and their combination increased glucose uptake in the liver and kidney, whereas that into the muscle and adipose tissue was not affected; this was accompanied by reductions in liver triglyceride content with both monotherapies and their combination (Klein et al. 2014).

In an acute OGTT in young Zucker diabetic fatty rats, the combination of linagliptin and empagliflozin (both at 1 mg/kg) additively decreased glucose excursion (AUC reduction: linagliptin −40 %, empagliflozin −25 %, combination −68 %); similar results were obtained in non-diabetic C57Bl/6 mice (Thomas et al. 2011). In another study, the effects of 15 days of treatment with empagliflozin (1 mg/kg), pioglitazone (10 mg/kg), or their combination were compared to those of vehicle in Zucker diabetic fatty rats (Thomas et al. 2013). While blood glucose level increased by 18.6 % in the control group, it decreased by 36.9 and 48.2 % vs. control with empagliflozin and pioglitazone monotherapy, respectively, and by 75.7 % with the combination. At the end of the study, fed blood glucose levels were 26.7, 16.9, and 13.8 mM in the control, empagliflozin, and pioglitazone groups, respectively, and 6.5 mM in the combination group. HbA_{1c} increased from 3.19 to 5.79 % between day 0 and day 15 in the control group. The increase in HbA_{1c} was less in the empagliflozin and in the pioglitazone group than in the control group, while the combination group had the lowest increase. Taken together, these combination studies demonstrated that beneficial metabolic effects of empagliflozin are additive when combined with other types of anti-hyperglycemic treatments including linagliptin and pioglitazone.

Studies of glucose homeostasis in type 1 diabetes

Empagliflozin has also been tested in several animal models of type 1 diabetes. Most of them are based on injection of a single high dose (50–60 mg/kg) of STZ, a naturally occurring glucosamine-nitrosourea causing ADP-ribosylation; because it is a substrate of the glucose transporter GLUT2, its toxicity preferentially destroys the insulin-producing β-cells of the pancreas. Such injections initially produce a phase of light diabetes, followed later by a more severe increase in blood glucose concentrations.

In an acute study, 1 week after STZ injection, fasted rats received two doses of insulin glargine with or without empagliflozin and were monitored for 6 h (Luippold et al. 2012). The lowest glucose levels were observed about 1 h after injection and were 15, 11, and 9 mM in rats given 1.5 IU insulin, 6 IU insulin, and 1.5 IU insulin plus 10 mg/kg empagliflozin, respectively. Accordingly, the 6-h AUC for blood glucose was reduced by 21.7, 44.1, and 49.1 %, respectively. In a second acute study, the investigators administered saline or low-dose insulin to non-fasted rats followed 2 h later by 10 mg/kg empagliflozin or vehicle and measured blood glucose for another 6 h (Luippold et al. 2012). In rats receiving only insulin, glucose gradually recovered from the lowest value at 1 h and approached levels of saline-injected rats. However, glucose remained close to the 1-h level for the entire observation period in rats receiving insulin followed by empagliflozin. In a third acute study, similar observations were made in rats receiving high-dose insulin followed by empagliflozin 3 h later (Luippold et al. 2012). Finally, these investigators performed a subchronic study in which rats received one or two insulin implants; starting 2 days later, rats received 10 mg/kg empagliflozin twice daily for 28 days (Luippold et al. 2012). At study end, the calculated 12-h blood glucose profile was similarly lowered by a single insulin implant or empagliflozin monotherapy; combination of both yielded a blood glucose lowering close to that of two insulin implants (Fig. 5). HbA_{1c} levels mimicked these findings.

Dose-dependent improvement of blood glucose by 10 and 30 mg/kg empagliflozin in a rat STZ model of type 1 diabetes was also confirmed in a 7-week study (Oelze et al. 2014). Empagliflozin has also been studied in rats maintained on a high-fat diet and receiving a single lower dose (35 mg/kg) of STZ (Vickers et al. 2012). One week after injection of STZ, rats were allocated to vehicle, empagliflozin (10 mg/kg orally once daily), or glucagon-like peptide-1 agonist exenatide (30 μg/kg/day via subcutaneous minipumps). As compared to vehicle, empagliflozin treatment improved fasting glucose (6.6 vs. 10.7 mM) and glucose control during an OGTT as assessed on day 23 (AUC 24.2 vs. 52.2 mM h) and also reduced plasma glucose (7.6 vs. 15.7 mM), HbA_{1c} (7.6 vs. 9.0 %), insulin (0.7 vs. 1.6 ng/ml), and triacylglycerol (0.21 vs. 0.36 mM) as assessed on day 29; in contrast, exenatide reduced triacylglycerol to 0.28 mM but did not affect any of the other metabolic parameters at either time point.

Akita ($Ins2^{+/C96Y}$) mice are a non-obese insulin-dependent model of spontaneous type 1 diabetes. The effects of empagliflozin (300 mg/kg of diet for 15 weeks; corresponding to 60–80 mg/kg/day) were explored in these mice, using littermate $Ins2^{+/+}$ mice as controls (Vallon et al. 2014). In contrast to STZ-treated animals (Vallon et al. 2013), Akita mice, empagliflozin-treated controls, and empagliflozin-treated Akita mice exhibited an up-regulated renal membrane SGLT2 expression; this was accompanied by a reduced expression of SGLT1. Facilitated by these changes in SGLT expression, empagliflozin strongly lowered blood glucose in Akita (glucose levels: control 100–140, Akita 517–535, empagliflozin-treated Akita 187–237 mg/dl).

An interesting addition to the data with empagliflozin in models of type 1 diabetes are mouse models which have genetically been engineered to lack SGLT2 (Vallon et al. 2013) or were selected for a normoglycemic glucosuria phenotype upon mutagen exposure that was

due to a non-sense mutation of the SGLT2-encoding gene Slc5a2, the Sweet Pee mouse (Ly et al. 2011). Diabetes was induced in both strains by STZ injection. The knockout mice exhibited normal renal mRNA expression of glucose transporters SGLT1, NaGLT1, GLUT1, or GLUT2 in response to STZ but a smaller increase in blood glucose levels (to approximately 300 vs. 470 mg/dl) (Vallon et al. 2013). Prior to diabetes induction, Sweet Pee mice exhibited similar HbA_{1c} as wild-type animals (Ly et al. 2011). Upon a first STZ injection, 86 % of wild-type but only 52 % of homozygous Sweet Pee mice became diabetic. In diabetic animals, similar to the effects of empagliflozin, homo- and heterozygous Sweet Pee mice exhibited lower HbA_{1c} as measured 16 weeks after diabetes induction. STZ-diabetic Sweet Pee mice exhibited a higher risk for urinary tract infection and an increased mortality rate (70 % in homozygous mutants vs. 10 % in controls at 20 weeks). On the other hand, no increase in mortality had been observed in STZ-treated SGLT2 knockout mice (Vallon et al. 2013) or in empagliflozin-treated Akita mice (Vallon et al. 2014). The reasons for these differences remain to be determined but may in part be due to the higher dose of STZ given to the Sweet Pee mice vs. their controls.

Obesity and weight loss

Obesity often is the basis for type 2 diabetes development; many existing medical treatments for hyperglycemia often lead to further body weight gain, thereby creating a vicious circle. In contrast, type 1 diabetes often is associated with low body weight or even weight loss. Against this background, the effects of empagliflozin on body weight have been explored in non-obese mice and in animal models of obesity and type 2 and type 1 diabetes.

Normoglycemic, non-obese wild-type and SGLT1 knockout mice were treated with empagliflozin (300 mg/kg diet) for 3 weeks (Rieg et al. 2014). In line with the promotion of glucose excretion, empagliflozin reduced body weight in both strains by approximately 1.5 and 3 %, respectively, despite an increase in food and water intake. In corroboration of these findings, an approximately 10 % reduction of body weight and increases in food and water intake were observed in SGLT1/2 double knockout mice in that study. Another study from the same group performed in wild-type mice reported that the effect of increased urinary glucose excretion induced by a 15-week treatment with empagliflozin was partly offset by increased food and fluid intake but nevertheless resulted in an overall modest weight loss associated with a reduction in epididymal white adipocyte size (Vallon et al. 2014).

In euglycemic rats with diet-induced obesity, a 4-week empagliflozin treatment (3 and 10 mg/kg) reduced body weight by 4.1 and 6.9 %, respectively, without affecting daily food intake (Grempler et al. 2010). At least for the higher dose, this was accompanied by a reduction in body fat content (from 175 ± 5 to 152 ± 3 g/rat), whereas body water or protein content were not altered; indicating that a loss of fat accounted for most of the body weight reduction. A follow-up study of similar design confirmed the effect on glucose excretion but reported a somewhat smaller reduction in body weight (−3.1 %); on the other hand, empagliflozin largely abolished the weight gain induced by pioglitazone in this model (+1.6 vs. +6.8 %) (Grempler et al. 2011). In a more detailed study, rats were exposed to a cafeteria-style diet for 15–20 weeks to induce obesity; thereafter, two treatment studies were initiated (Vickers et al. 2014). In the first study, rats were treated with vehicle or 10, 30, or

60 mg/kg empagliflozin for 28 days. In the second study, they received 10 mg/kg empagliflozin in combination with either once daily 5 mg/kg of the serotonin-noradrenaline uptake inhibitor sibutramine or twice daily 20 mg/kg of the lipase inhibitor orlistat. Empagliflozin dose-dependently increased urinary glucose excretion and reduced body weight, plasma leptin, and body fat. The combination of empagliflozin and orlistat reduced body weight compared to animals treated with either drug alone and improved glucose tolerance, plasma insulin, and leptin compared to vehicle-treated controls. The effect of sibutramine to improve glycemic control in an oral glucose tolerance test was also increased with empagliflozin, and combination treatment led to a reduction in carcass fat greater than that observed with either drug alone. Taken together, these studies established that empagliflozin can reduce body weight in non-obese and obese rodents; this occurs due to a loss of body fat, probably secondary to enhanced glucose excretion, and is additive to known weight loss medications. Studies in the Sweet Pee mice have reported a reduced body weight gain over time with an intermediate effect in heterozygous animals (Ly et al. 2011). In non-diabetic SGLT2 knockout mice, the situation is less clear as reduced (Jurczak et al. 2011) and normal body weight have been reported (Vallon et al. 2013; Powell et al. 2013).

During a 7-day treatment of db/db mice with empagliflozin, 24-h water intake was transiently increased on day 1; while food intake was not altered during the entire study period, body weight gain was lowered on days 1 to 5 (Lin et al. 2014). In a second experiment with a 10-week empagliflozin treatment, body weight of db/db mice during the final 4 weeks of the study was slightly higher in empagliflozin- than in vehicle-treated rats; empagliflozin also had only minor if any effects on food and water intake at these time points.

In another study, rats were administered vehicle or low-dose STZ (35 mg/kg) and maintained on a high-fat diet for 29 days (Vickers et al. 2012). After 1 week, the animals were allocated vehicle, empagliflozin (10 mg/kg orally), or exenatide treatment (30 μg/kg/day via subcutaneously implanted minipumps). At study end, in comparison to vehicle-treated rat, STZ-treated animals exhibited the expected decrease in body weight (519 vs. 568 g) and plasma insulin (1.6 vs. 2.8 ng/ml) and an increase in HbA_{1c} (9 vs. 7 %) and plasma glucose (15.7 vs. 8.3 mM) and triacylglyceride levels (0.36 vs. 0.2 mM). Empagliflozin treatment did not affect body weight or food intake compared with STZ-treated controls but improved metabolic parameters (see “Studies of glucose homeostasis in type 1 diabetes” Section). Unaltered food intake possibly reflected unchanged urinary glucose excretion in steady-state, i.e., inhibition in renal glucose reabsorption was balanced by reduced glucose filtration as observed in empagliflozin-treated Akita mice (Vallon et al. 2014) and STZ-diabetic SGLT2 knockout mice (Vallon et al. 2013).

Weight loss is one potential symptom in patients with type 1 diabetes at the onset of the disease or in inadequately controlled glucose homeostasis. In a rat STZ model, empagliflozin did not reduce but rather increase body weight although to a lesser extent than one or two insulin implants (+7.5 vs. +11.1 and +20.0 %, respectively) (Luippold et al. 2012). In contrast to insulin treatment, empagliflozin did not increase body fat content. The reduction in body weight in the STZ model of type 1 diabetes was similar in wild-type and SGLT2 knockout mice when assessed 5 weeks or 4.5 months after diabetes induction

(Vallon et al. 2013). The Akita model of type 1 diabetes exhibits a reduction of body weight over time; in this model, treatment with empagliflozin for up to 15 weeks attenuated this reduction and the reduction in epididymal adipocyte size despite a similar increase in food and fluid intake compared with vehicle treatment (Vallon et al. 2014).

Taken together, these data demonstrate that, in contrast to many other anti-hyperglycemic medications, empagliflozin reduces body weight in animal models of obesity, irrespective of the presence of concomitant diabetes. In contrast, empagliflozin treatment or SGLT2 knockout do not enhance the disease-associated body weight reduction in animal models of type 1 diabetes. In clinical studies with type 2 diabetes patients, empagliflozin treatment also caused a reduction in body weight relative to placebo (Häring et al. 2013; Häring et al. 2014; Kovacs et al. 2014) or the dipeptidylpeptidase 4 inhibitor sitagliptin (Roden et al. 2013).

Cardiovascular consequences of diabetes

Cardiovascular complications are an important cause of morbidity and mortality in patients with diabetes, and endothelial dysfunction is an early indicator of developing cardiovascular complications. Using a rat STZ model of type 1 diabetes, the effect of a 7-week treatment with empagliflozin (10 and 30 mg/kg) on endothelial dysfunction has been studied (Oelze et al. 2014). Such treatment lowered FPG from ≈400 to 170–185 mg/dl and normalized endothelial dysfunction as assessed by endothelium-dependent relaxation of aortic rings, and reduced oxidative stress (Fig. 6) in these vessels as well as vascular wall thickness and collagen content; moreover, empagliflozin normalized the elevated NADPH-oxidase activity in the heart. Endothelial dysfunction was also improved in the aorta of db/db mice following a 10-week empagliflozin treatment, along with a reduction of elevated aortic superoxide levels (Lin et al. 2014).

Diabetes can also lead to arterial stiffness, which in turn may increase the risk for cardiovascular complications. db/db mice exhibit cardiac interstitial and pericoronary aterial fibrosis together with coronary arterial thickening, interstitial macrophage infiltration, and elevated cardiac superoxide levels; all of these changes were normalized by a 10-week empagliflozin treatment. In line with these morphological findings in a type 2 diabetes mouse model, an 8-week treatment of young and normotensive type 1 diabetes patients with empagliflozin reduced augmentation indices (Cherney et al. 2014a), an index of arterial stiffness which is associated with cardiovascular risk (Nürnberger et al. 2002), at the radial, carotid, and aortic positions under clamped euglycemic conditions; similar effects were seen under clamped hyperglycemic conditions. Empagliflozin concomitantly decreased carotid-radial pulse wave velocity under euglycemic and hyperglycemic conditions, while declines in carotid-femoral pulse wave velocity were only observed during clamped hyperglycemia (5.7 ± 1.1 to 5.2 ±0.9 m/s); in that study, reported separately (Perkins et al. 2014), HbA_{1c} was reduced from 8.0 to 7.6 %. The authors speculated that this decline in arterial stiffness in young type 1 diabetes mellitus subjects may relate to pleiotropic actions of SGLT2 inhibition, including glucose lowering, anti-hypertensive, and weight reduction effects.

Arterial hypertension often co-exists with diabetes, and the two conditions can enforce each other with regard to long-term adverse effect on the cardiovascular system including renal outcomes. In euglycemic, normotensive wild-type mice, a 15-week treatment with

empagliflozin did not affect blood pressure, heart rate, or plasma aldosterone concentrations (Vallon et al. 2014). Empagliflozin treatment for 12 weeks did not affect blood pressure in the BTBR ob/ob model of type 2 diabetes, irrespective of whether these mice were normotensive or hypertension had been induced by infusion of angiotensin II (Gembardt et al. 2014). Empagliflozin treatment for 10 weeks also did not affect blood pressure in db/db mice as assessed at the 3- or 8-week time point (Lin et al. 2014). While the Akita mouse model of type 1 diabetes exhibited a similar heart rate as wild-type mice, it was characterized by increased blood pressure and plasma aldosterone (Vallon et al. 2014); in such mice, empagliflozin did not affect heart rate or aldosterone but normalized blood pressure. Blood pressure lowering was also reported upon 18 weeks of treatment with empagliflozin (10 mg/kg/day) in a study with Cohen Rosenthal diabetic hypertensive rats (Younis et al. 2014b). In that study, empagliflozin treatment also reduced left ventricular mass and end-diastolic diameter; left ventricular end-systolic diameter, and fractional shortening also tended to be improved.

In a study with young, normotensive type 1 diabetes patients, an 8-week treatment with empagliflozin caused a small reduction in systolic blood pressure (111 ± 9 to 109±9 mmHg) as assessed during clamped euglycemia (Cherney et al. 2014a); heart rate, heart rate variability, and plasma noradrenaline and adrenaline remained unchanged. Possible effects on blood pressure were also explored in a pooled analysis of two placebo-controlled phase 2 studies in type 2 diabetes patients, in which 10 or 25 mg empagliflozin once daily or placebo was administered for 12 weeks as monotherapy or as add-on to metformin (n = 152–153 per group) (Hach et al. 2012). Observed systolic blood pressure reductions were 1.2, 3.8, and 4.5 mmHg with placebo and 10 and 25 mg empagliflozin, respectively; corresponding reductions in the subgroup of patients with a baseline systolic pressure of >140 mmHg were 10.4, 17.0, and 13.4 mmHg, respectively. The extent of blood pressure lowering was not correlated to that of HbA_{1c} reduction. Blood pressure reductions relative to placebo or sitagliptin treatment have also been observed in response to empagliflozin in phase III studies, in which reductions within the hypertensive subgroup were greater than in the overall population (Roden et al. 2013; Häring et al. 2013, 2014; Kovacs et al. 2014; Tikkanen et al. 2015). The modest initial natriuresis and the preserved osmotic diuresis induced by empagliflozin may contribute to these blood pressure reductions.

In the above animal and human studies, empagliflozin in therapeutic doses had no effects on heart rate. This lack of effect was also observed with supra-therapeutic doses up to 800 mg in a single rising dose study in healthy volunteers (Seman et al. 2013). Moreover, neither a therapeutic (25 mg) nor a supra-therapeutic dose (200 mg) of empagliflozin affected mean change from baseline for heart rate-corrected QT interval in healthy volunteers (Ring et al. 2013).

Plasma cholesterol, specifically LDL cholesterol, is a cardiovascular risk factor similar to diabetes. While no direct effects of SGLT2 inhibitors on plasma cholesterol are expected based on its mode of action, modulation by indirect mechanisms may occur. Thus, treatment with SGLT2 inhibitors as a class was associated with small increases in LDL cholesterol in clinical studies as reviewed elsewhere (Halimi and Verges 2014). A pooled analysis of four clinical phase III studies with empagliflozin reported increases in both LDL and HDL

cholesterol (mean change from baseline total cholesterol 1.5, 4.2, and 6.2 mg/dl; LDL cholesterol 0.8, 3.1, and 3.9 mg/dl; HDL cholesterol 0.0, 2.7, and 2.7 mg/dl for placebo; 10 and 25 mg empagliflozin, respectively); the LDL/HDL ratio was not affected by either dose (Hach et al. 2013). Our search has identified only one pre-clinical study in which the effect of empagliflozin has been assessed; in that study, in rats with STZ-induced diabetes, empagliflozin did not elevate but rather lower plasma cholesterol, an effect reaching statistical significance at the higher dose (105 ± 8 vs. 93 ± 4 vs. 83 ± 5 mg/dl for placebo, 10 and 30 mg/kg/day empagliflozin, respectively) (Oelze et al. 2014). SGLT2 knockout mice did not exhibit significant alterations of plasma cholesterol in the absence or presence of STZ-induced diabetes (188 ± 37 vs. 186 ± 45 and 173 ± 32 vs. 202 ± 60 mg/dl, respectively) (Powell et al. 2013). The clinical relevance of these findings will be determined in the ongoing cardiovascular outcome study.

db/db mice exhibit cardiac interstitial and pericoronary aterial fibrosis together with coronary arterial thickening, interstitial macrophage infiltration, and elevated cardiac superoxide levels; all of these changes were normalized by a 10-week empagliflozin treatment which caused a marked reduction of plasma glucose levels (Lin et al. 2014).

Renal consequences of diabetes

Nephropathy is another important complication of diabetes (Stanton 2014), and primary changes in the proximal tubular system have been implicated in the early and late consequences observed in the diabetic kidney (Vallon 2011; Vallon and Thomson 2012). One study has examined whether empagliflozin affects the response of a human proximal tubule cell line, HK2 cells, to high glucose (Panchapakesan et al. 2013). HK2 cells were exposed to control glucose (5 mM) and high glucose (30 mM). High glucose increased nuclear DNA binding for nuclear factor κB (NF-κB) and activator protein 1, induced expression of Toll-like receptor-4 and collagen IV, and enhanced interleukin-6 secretion; empagliflozin attenuated all of these effects. As empagliflozin did not reduce NF-κB expression induced by high mobility group box protein 1, its effect may be specifically related to a reduction in glucotoxicity. Taken together, these results indicated that in human proximal tubule cells in culture, empagliflozin reduced the high glucose-induced activation of pathways that have been implicated in inflammation and fibrosis.

Another study has investigated effects of SGLT2 blockade by using siRNA in early passages of cultured human proximal tubule cells (Maeda et al. 2013). The siRNA construct used in these studies fully suppressed immunodetectable SGLT2 expression and cellular glucose entry. Moreover, it inhibited high glucose-induced reactive oxygen species generation and expression of the advanced glycation end product receptor. High glucose augmented the pro-apoptotic response to advanced glycation end products; such enhancement was also inhibited by SGLT2 siRNA. These data raise the possibility that SGLT2 inhibition may protect tubular cells against high glucose-induced apoptosis, but the in vivo relevance of studies in cell culture in the context of diabetic nephropathy remains to be determined.

To extend such findings to the in vivo level, two studies were performed in ob/ob mice which spontaneously develop type 2 diabetic nephropathy (Gembardt et al. 2014). In the first study, BTBR ob/ob mice received a diet containing 300 ppm empagliflozin or equicaloric

placebo chow for 12 weeks. In a second study, they received the same diets for 6 weeks but additionally osmotic minipumps releasing 1 μg/kg body weight/day angiotensin II. In both studies, empagliflozin reduced blood glucose by approximately 140–150 mg/dl to end-of-study levels of 230 and 170 mg/dl, respectively, and concomitantly reduced albuminuria. While empagliflozin treatment did not affect matrix expansion as evaluated by immunohistochemistry, it decreased the diabetes-related glomerular hypertrophy. In db/db mice, a 10-week treatment with empagliflozin improved the urinary albumin to creatinine excretion, glomerular sclerosis index, glomerular macrophage infiltration, and glomerular superoxide levels (Lin et al. 2014).

Empagliflozin effects on renal function and morphology were also tested in the Akita mouse model of type 1 diabetes (Vallon et al. 2014). In this study, empagliflozin reduced blood glucose levels from ~530 to 200 mg/dl. It modestly reduced glomerular filtration rate in controls (250 vs. 306 μl/min) and completely prevented the diabetes-induced increase in filtration rate (255 vs. 397 μl/min). Empagliflozin also attenuated increases in kidney weight and urinary albumin/creatinine ratio in Akita mice in proportion to hyperglycemia but did not increase urinary glucose/creatinine ratios, indicating that the reduction in filtered glucose balanced the inhibition of glucose reabsorption (Fig. 7). Moreover, empagliflozin attenuated/prevented the increase in glomerular size and molecular markers of kidney growth, inflammation, and gluconeogenesis in Akita mice.

A previous study had induced diabetes by low-dose STZ in SGLT2 knockout mice and explored markers of renal growth and injury 5 weeks and 4.5 months after STZ administration (Vallon et al. 2013). SGLT2 knockout reduced blood glucose in STZ-diabetic mice from 470 to 300 mg/dl, i.e., blood glucose levels remained higher compared to the empagliflozin-treated Akita mice (Vallon et al. 2014); see the latter paper for potential explanations. Increases in glucosuria and food and fluid intake were similar in both strains. Lack of SGLT2 prevented STZ-induced glomerular hyperfiltration but not the increase in kidney weight. Additional experiments showed that the reduction in GFR occurred independent of lowering blood glucose. SGLT2 knockout attenuated the STZ-induced renal accumulation of p62/sequestosome, an indicator of impaired autophagy, but did not attenuate the rise in renal expression of markers of kidney growth, oxidative stress, inflammation, fibrosis, or injury. SGLT2 deficiency did not induce ascending urinary tract infection in non-diabetic or diabetic mice. These studies in empagliflozin-treated Akita and STZ-diabetic SGLT2 knockout mice confirmed that SGLT2 inhibition can lower GFR independent of reducing blood glucose. This is consistent with the tubular hypothesis of diabetic glomerular hyperfiltration, and the original observation that an increase in SGLT-mediated sodium transport contributes to the diabetes-induced proximal tubular hyperreabsorption, which lowers the Na-Cl-K concentration at the macula densa and increases GFR through the physiology of tubuloglomerular feedback (Vallon et al. 1999). In contrast to the GFR effect, SGLT2 inhibition appears to attenuate albuminuria, kidney growth, and inflammation in the early diabetic kidney mostly secondary to lowering blood glucose (Vallon et al. 2014).

Effects of empagliflozin were also tested in endothelial NO synthase knockout mice which had been injected with a low dose of STZ (Komala et al. 2014). In that study, diabetic mice

received injections of insulin glargine thrice weekly whenever their blood sugar reading was higher than 28 mM or if they had lost weight more than 25 % from their pre-diabetic state resulting in an average blood glucose concentration of 21–22 mM in diabetic mice, irrespective of empagliflozin treatment; this design was chosen to further explore possible nephroprotection independent of glucose lowering. Under these conditions, empagliflozin treatment did not improve albuminuria, glomerulosclerosis, tubular atrophy, tubulointerstitial macrophage infiltration, or interstitial fibrosis; in contrast, the angiotensin receptor antagonist telmisartan, used as positive control, improved or even normalized all of these parameters.

Nephroprotective effects of empagliflozin have also been explored in rats. Reduced albuminuria was reported upon 18 weeks of treatment with empagliflozin (10 mg/kg/day) in a study with Cohen Rosenthal diabetic hypertensive rats (Younis et al. 2014b). Within that study, empagliflozin treatment also prevented the adipose tissue and fat accumulation in the pancreas (Younis et al. 2014a). In other studies, Dahl salt-sensitive rats were fed a high-salt diet and additionally received an STZ injection to induce a hypertensive type 1 diabetes model (Murphy et al. 2014). In another study from the same investigators, Goto-Kakizaki rats were implanted with a long-acting deoxycorticosterone acetate pellet and thereafter received drinking water containing 1 % NaCl, a hypertensive type 2 diabetes model (Murphy et al. 2014). In both studies, rats were allocated to receive empagliflozin (20 mg/kg/day), the angiotensin-converting enzyme inhibitor lisinopril (10 mg/kg/day), or their combination or were subcutaneously implanted with Silastic insulin pellets for 8 (type 1) or 12 weeks (type 2 diabetes). In the type 1 diabetes study, blood glucose levels were 400–500 mg/dl in the vehicle and lisinopril groups and 100–150 mg/dl in the insulin, empagliflozin, and empagliflozin + lisinopril groups; combination treatment lowered systolic blood pressure to a much greater extent than monotherapy or insulin. In this model, lisinopril had only little effect on proteinuria, while empagliflozin reduced it; combination treatment provided a much greater reduction, almost reaching the level seen with insulin. While neither monotherapy improved the histologically determined glomerular injury score or renal outer medullary fibrosis, both parameters were improved upon combination treatment. In the type 2 diabetes model, blood glucose levels were 200–300 mg/dl in the vehicle and lisinopril groups and 100–150 mg/dl in the insulin, empagliflozin, and empagliflozin + lisinopril groups; both monotherapies improved the glomerular injury score and cortical fibrosis, but combination treatment was not more effective; other than insulin, none of the treatments affected medullary fibrosis. These data extend potential nephroprotective effects of empagliflozin to hypertensive rat models of type 1 and 2 diabetes; they also indicate that such nephroprotection may exist on top of known effective agents such as converting enzyme inhibitors.

Taken together, these studies demonstrate that empagliflozin attenuates or even abolishes complications of type 1 or type 2 diabetes in the vasculature and kidney, unless given under conditions where it does not improve diabetes. While additional experimental studies on effects of empagliflozin and other SGLT2 inhibitors on diabetic complications are desirable, it is interesting to note that a clinical study with 8-week treatment of type 1 diabetes patients with empagliflozin, in line with the above animal studies, has reported attenuation of renal hyperfiltration (Cherney et al. 2014b). An exploratory post-hoc analysis of these data

showed that empagliflozin increased 24-h fractional sodium excretion in patients with renal hyperfiltration but not those without (Skrtic et al. 2014). During clamped euglycemia, patients with hyperfiltration exhibited lower afferent arteriole resistance and higher estimated glomerular pressure and filtration pressure across the glomerular membrane as compared to those without hyperfiltration while estimated efferent glomerular resistance and glomerular oncotic pressure were similar between groups; in response to empagliflozin, afferent resistance increased and intraglomerular pressure and filtration pressure decreased in patients with hyperfiltration but remained unchanged in patients without hyperfiltration. While hyperfiltration has been proposed to facilitate the development of chronic kidney disease (Magee et al. 2009), its improvement by empagliflozin apparently occurs by a different mechanism than by angiotensin-converting enzyme inhibitors or angiotensin II type 1 receptor antagonists (Vallon et al. 1999; Stanton 2014); therefore, it will be interesting to explore how the combination of empagliflozin with an inhibitor of the renin-angiotensin system will affect the decline of renal function in animal models or patients with diabetes.

Other in vivo effects

Sixteen-week-old db/db mice exhibited impaired learning and reference/working memory as compared with age-matched db/m mice as assessed in a Morris water maze test; these were improved by a 10-week empagliflozin treatment (Lin et al. 2014). This empagliflozin-induced cognitive improvement was accompanied by reductions in cerebral superoxide, oxidative DNA damage, and expression of NADPH oxidase subunit expression as well as an increase in expression of brain-derived neurotrophic factor.

Conclusions

The data summarized in this article demonstrate that empagliflozin is a potent and selective SGLT2 inhibitor. In line with data from mice carrying non-sense mutations of the SGLT2-encoding genes or SGLT2 knockout mice, treatment with empagliflozin promotes urinary glucose excretion, which leads to a lowering of blood glucose, smaller glucose excursions during oral glucose tolerance tests, and, upon chronic treatment, a reduction of HbA_{1c}. In contrast to classic antihyperglycemic medications such as insulin, sulfonylureas, and thiazolidinediones (Purnell and Weyer 2003), empagliflozin leads to body weight reductions in obese and type 2 diabetic animal models and patients with type 2 diabetes. Moreover, it is associated with modest blood pressure lowering in animals and patients, without changes in heart rate. In animal models, the blood glucose lowering effect of empagliflozin has beneficial effects on vascular and renal complications of diabetes. Whether these cardiovascular and renal effects observed in animal and/or short-term human studies translate into long-term clinical benefits in patients is currently under investigation (Zinman et al. 2014).

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Abbreviations

AUC	Area under the curve
OGTT	Oral glucose tolerance test
FPG	Fasting plasma glucose
NF-κB	Nuclear factor κB
SGLT	Sodium glucose transporter
STZ	Streptozotocin

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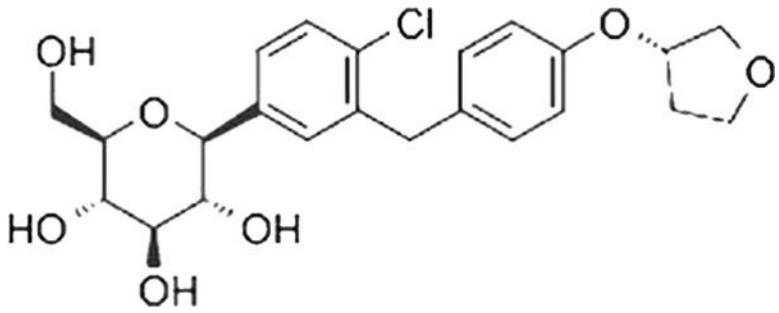

Fig. 1.

Chemical structure of the C-glucoside empagliflozin (BI 10773; 1-chloro-4-(*β*-D-glucopyranos-1-yl)-2-[4-((*S*)-tetrahydrofuran-3-yl-oxy)-benzyl]-benzene). Taken from US prescribing information (www.jardiance.com)

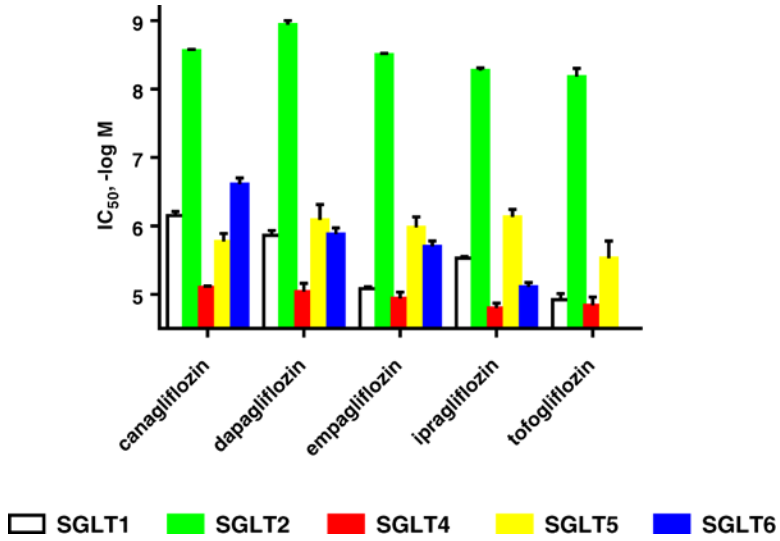

Fig. 2.

Potency of empagliflozin and C-glucoside comparator compounds for inhibition of human SGLTs expressed in HEK cells. Data are means ± SEM. Created using data from Grempler et al. (2012b)

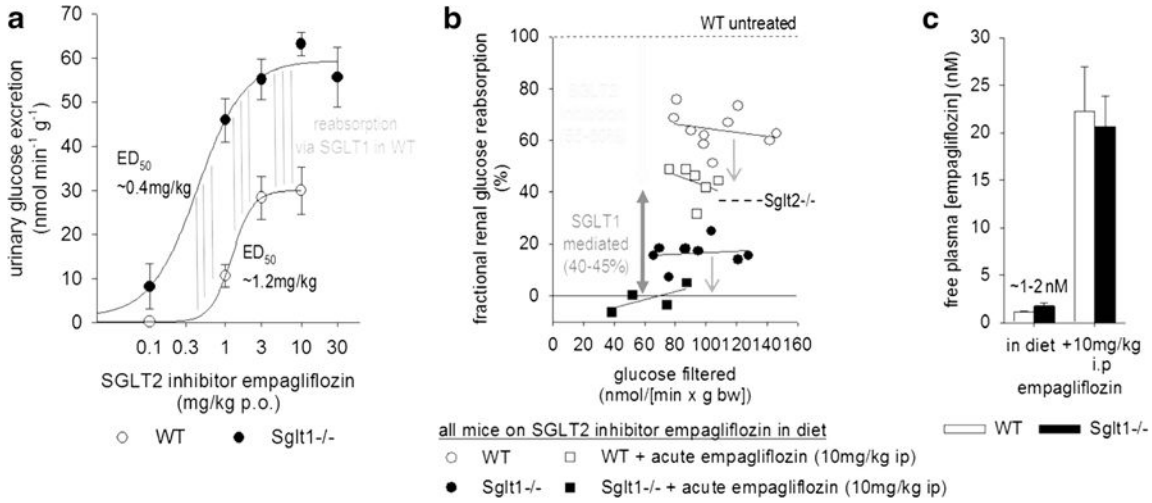

Fig. 3.

Empagliflozin-induced glucosuria in normoglycemic mice. **a** In metabolic cages, acute oral application of empagliflozin dose-dependently increased urinary glucose excretion in wild-type mice (WT). Compared with WT, the empagliflozin-induced glucosuric response was shifted leftward and the maximum response doubled in mice lacking SGLT1 ($Sglt1^{-/-}$). The difference between dose response curves, which reflects the glucose reabsorption mediated via SGLT1 in WT mice, reached a maximum at 0.4 mg/kg (indicated at the *left of the vertical lines*) and was maintained (all vertical lines have same length) for higher doses up to 10 mg/kg, indicating a high selectivity of empagliflozin vs. SGLT1 in this dose range. Note that empagliflozin began to increase glucose excretion in WT when reabsorption via SGLT1 reached its maximum. **b** Fractional renal glucose reabsorption (FGR) was determined in inulin clearance studies following empagliflozin treatment (300 mg/kg of diet) for 3 weeks. Each *dot* represents 1 clearance experiment period. **c** Free plasma concentrations of empagliflozin, corresponding to early tubular concentrations, were similar to reported IC_{50} for mouse SGLT2 (1–2 nM), when the drug was given “in the diet” only. Additional application of empagliflozin 1 h before the study increased free plasma concentrations to 20–22 nM and reduced FGR in WT to values of 40–45 % (similar to FGR reported in $Sglt2^{-/-}$) and completely prevented renal glucose reabsorption in $Sglt1^{-/-}$ mice. Taken from Rieg et al. (2014)

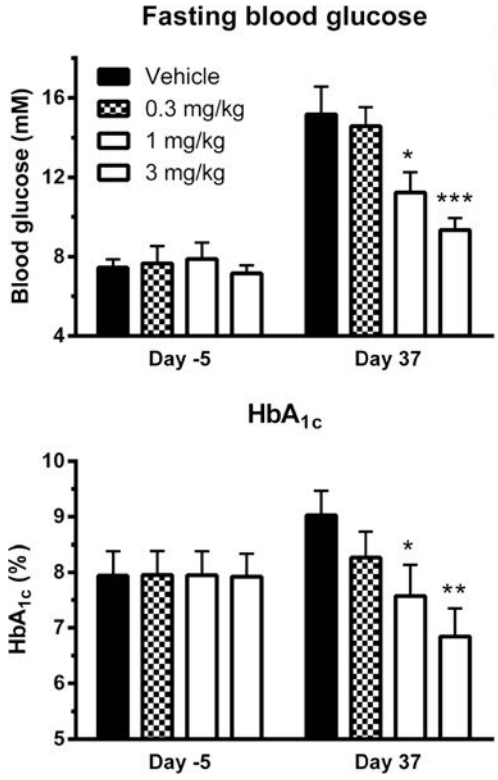

Fig. 4.

Chronic empagliflozin treatment dose-dependently improves fasting blood glucose (*upper panel*) and HbA_{1c} (*lower panel*) in 12-week-old Zucker rats, a model mimicking the course of type 2 diabetes. Data represent basal (study day −5) and end of treatment (study day 37) and are adapted with permission from Thomas et al. (2012)

*: $p<0.05$, **: $p<0.01$ and ***: $p<0.001$ vs. vehicle

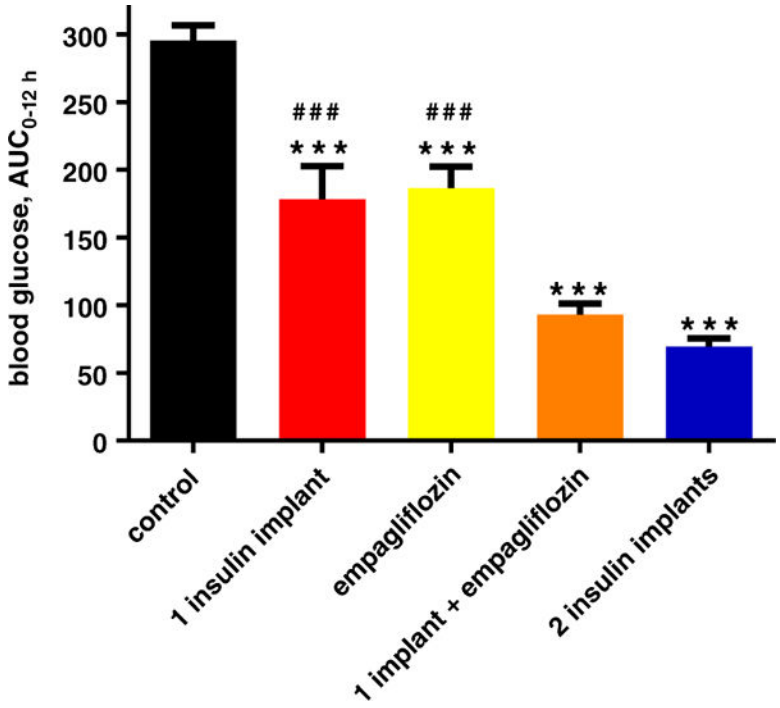

Fig.5.

Effects of treating rats with STZ-induced diabetes, a model of type 1 diabetes, for 28 days with a single insulin implant, empagliflozin (10 mg/kg twice daily), their combination, and two insulin implants on blood glucose concentrations. Data are shown as calculated 12-h blood glucose profiles ($AUC_{0–12\ h}$, mM h). $^{***}P < 0.001$ vs. control and $^{\#\#\#}P < 0.001$ vs. combination. Created using data from Luippold et al. (2012)

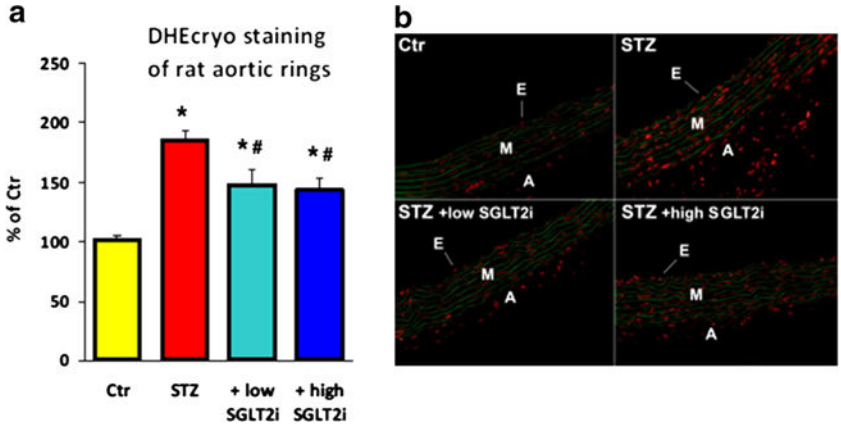

Fig. 6.

Effects of empagliflozin treatment on oxidative stress parameters in aorta of STZ-diabetic rats. Vascular oxidative stress was assessed by dihydroethidine (DHE, 1 μM)-dependent fluorescence microtopography in aortic cryosections. **a** Densitometric quantifications and **b** representative microscopic images are shown. SGLT2i: empagliflozin. Taken from Oelze et al. (2014)

*: $p < 0.05$ vs. control, #: $p < 0.05$ vs. STZ

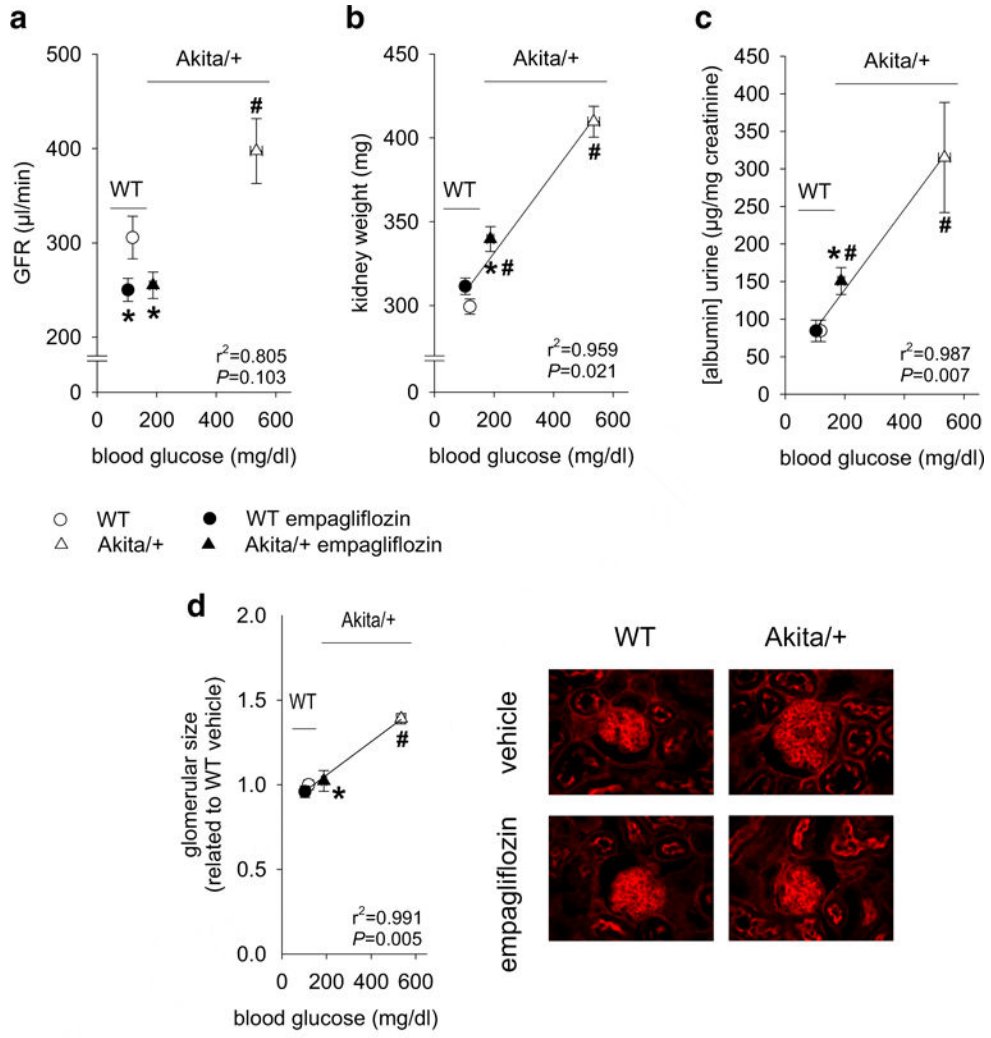

Fig. 7.

Empagliflozin prevented the diabetes-induced increase in glomerular filtration rate (GFR) and attenuated the increase in kidney weight, glomerular size, and albuminuria in proportion to hyperglycemia in **Akita**/+ mice, a model of type 1 diabetes. Empagliflozin (300 mg/kg of diet) or vehicle were given to **Akita**/$^{+}$ and wild-type (WT) mice for 15 weeks. Depicted are results for **a** GFR, **b** kidney weight, **c** urinary albumin/creatinine ratios, and **d** glomerular size. *$P < 0.05$ vs. vehicle treatment in same genotype; #$P < 0.05$ vs. WT. ANOVA and unpaired Student's t test and linear regression analysis. Linear regression lines were included when statistical significance was achieved. Taken from Vallon et al. (2014)