Current Trends in Endocrinology
Vol. 7, 2014

Review

Renal glucose handling and the kidney as a target to anti-diabetic medication

Eugenio Cersosimo*

Department of Medicine, Division of Diabetes, University of Texas Health Science Center, 7703 Floyd Curl Drive, MC 7886, San Antonio, Texas, 78229-3900, USA

ABSTRACT

In this article a review of the fundamental concepts behind the normal physiology and the adaptation of glucose handling by the kidney in diabetes mellitus is provided. The main objective is a wide and comprehensive discussion of the events leading to the development of a new class of anti-hyperglycemic agents that act primarily by chemical inhibition of tubular glucose reabsorption. Relevant molecular pathways and transport systems involved in renal glucose kinetics and metabolism are described. The process of glomerular ultrafiltration with complete proximal tubular reabsorption of glucose is discussed in detail, together with the contribution to systemic glucose homeostasis of renal gluconeogenesis and glucose utilization. The "maladaptive" changes that occur in the tubular reabsorptive capacity in patients with diabetes mellitus, which lead to increased rates of glucose re-entry into the circulation, thus perpetuating the hyperglycemia are analyzed. With a brief historical perspective, the development and expansion of these new anti-diabetic agents, which are capable of lowering blood glucose by blocking the activity of the sodium-glucose co-transporters in the kidney, are examined. Finally, results of some critical pharmacological and pre-clinical investigational studies, as well as clinical efficacy and safety data available for the current sodium-glucose transporter 2 (SGLT-2) inhibitors are analyzed in detail. In conclusion, some suggestions as to the best patient profile and clinical conditions in which these anti-diabetic agents are more likely to be beneficial are proposed.

KEYWORDS: glycosuria, SGLT-2, tubular reabsorption, kidney and diabetes, type 2 diabetes therapy, transport inhibitors

INTRODUCTION

The importance of the kidney in glucose homeostasis has been recognized for many years [1, 2]. A series of observations indicating that the renal contribution to glucose regulation and counter-regulation might be of greater significance than previously anticipated were reported in recent years [3-9], which rekindled the interest in the role of the kidney in glucose metabolism. Simultaneous studies were conducted in experimental diabetic animals using phlorizin [10], a compound with anti-diabetic properties extracted from the root bark of apple tree and known to inhibit tubular glucose transport. The hypothesis that a nonselective SGLT inhibition could reduce the tubular capacity for glucose reabsorption (lower *TmG*), promote glycosuria even at lower plasma glucose concentrations (lower RTG) and thus, decrease blood glucose levels has been confirmed [10]. This principle was then advanced to patients with diabetes and the pharmacological development of new agents has accelerated and reached clinical relevance.

Glucose handling by the kidney includes free glomerular filtration with complete proximal

*Cersosimo@uthscsa.edu,
Eugenio.Cersosimo@uhs-sa.com

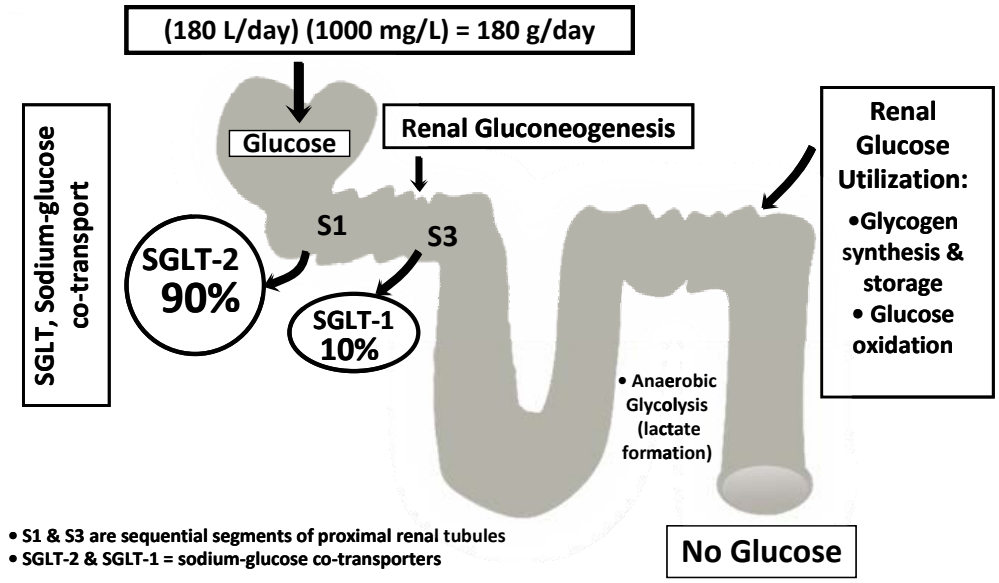

Figure 1. Schematic representation of the nephron showing the heterogeneity of glucose handling by the kidney. Plasma glucose is freely filtered at the glomerulus and completely reabsorbed in the proximal tubules. The SGLT-2 transporters located at the luminal membrane of cells in the S1 segment are responsible for 90% of total glucose re-uptake. The SGLT-1 transporters located downstream in the S3 segment of the proximal tubules account for the remaining 10% of the glucose load reabsorbed into the renal interstitial fluid. The process of renal gluconeogenesis occurs exclusively in the proximal tubular cells, whereas glucose utilization is limited to the distal nephron. Glycogen synthesis and storage, as well as complete glucose oxidation are detected only in cells of the distal nephron and, partial oxidation (anaerobic glycolysis) with formation and release of lactate is a characteristic of the hypoxic medullary regions of the kidney.

tubular reabsorption into the renal interstitial fluid space. Renal gluconeogenesis takes place in proximal tubular cells and adds a small fraction to the glucose in the interstitial fluid. After exchanging with proximal renal capillaries, glucose mixes with the venous blood and leaves the kidney into the renal vein. Glucose is extracted from the baso-lateral membrane in the distal nephron to be either stored in the form of glycogen (limited) or oxidized to generate energy. As a consequence, no glucose is excreted in the urine and essentially all filtered glucose load is restored to the peripheral circulation, after mixing with the remaining 80% of unfiltered blood in the renal circulation [11].

The evidence for the heterogeneity of glucose handling within the nephron (Figure 1) is substantiated by numerous reports in the literature [1, 12-15]. The sodium-glucose co-transport system (SGLT) is located at the luminal membrane of the proximal renal tubular cells [15], which also contains specific enzymes for glucose synthesis *de novo* (renal gluconeogenesis) [1, 12]. No enzymatic activity for glucose utilization, storage or oxidation has ever been identified in proximal tubules [14]. In contrast, the distal nephron and renal medulla have sufficient biochemical capability to metabolize glucose extracted from the peri-tubular fluid [13, 14]. The fact that most of the energy required by the tubular transport activity is supplied from fatty acid oxidation enables complete glucose sparing and represents a critical aspect in the maintenance of whole-body glucose homeostasis by the kidney.

Glucose handling by the kidney

Normal physiology

The transmembrane transport systems and intracellular metabolic pathways of glucose have been well characterized in the kidney. Most of the information is derived from *in vitro* cell preparations [16-18] and renal perfusion studies using experimental animal models [19-21]. Analogous

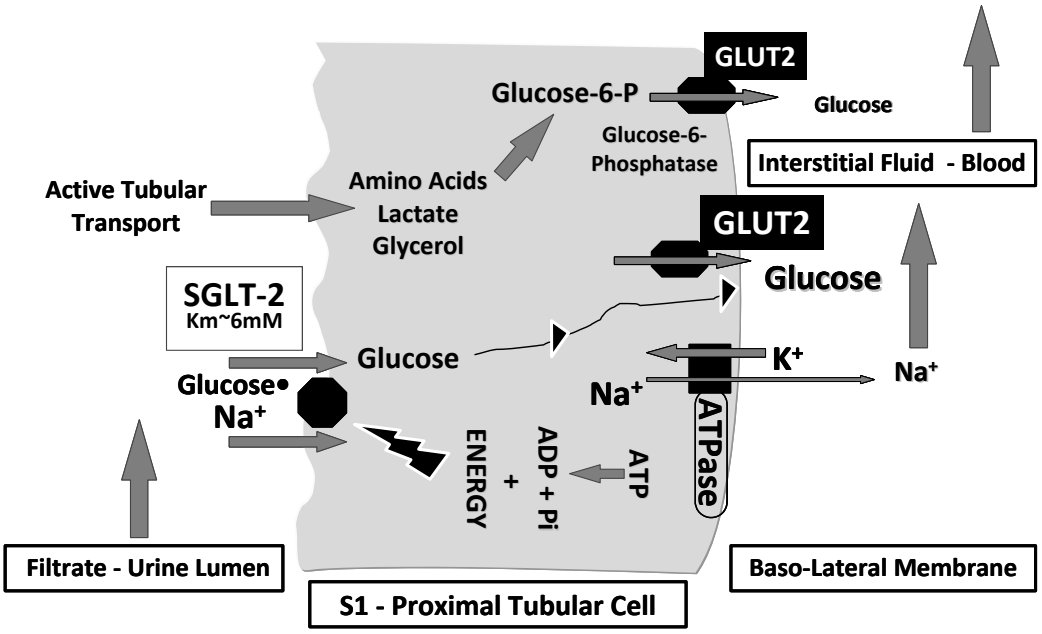

Figure 2. Diagram of a proximal tubular cell of the S1 segment showing transport activity and metabolic pathways that lead to effective sodium-glucose reabsorption and gluconeogenesis. Glucose arising in the glomerular filtrate enters the urine lumen, couples with sodium ions and binds avidly to the high capacity-low affinity (Km ~6.0 mM) SGLT-2 transporter. The energy required to actively transport the sodium-glucose complex into the cell is supplied by ATP generated by the sodium-potassium ATPase pump action, located in the baso-lateral membrane. Proximal tubules utilize primarily fatty acid oxidation to meet energy demands and are not capable of metabolizing glucose. Thus, glucose reaches the baso-lateral cell membrane intact, where the GLUT-2 transporter promotes a facilitated passive transport down its concentration gradient. Gluconeogenesis occurs from precursors such as amino acids, lactate and glycerol and the enzyme *glucose-6-phosphatase* enables dephosphorylation with release of glucose into the interstitial fluid mediated via the GLUT-2 facilitated transport. The sodium and glucose-rich proximal interstitial fluid exchanges with blood in the peri-tubular capillaries and perfuses the distal nephron.

findings have been reported in a few studies conducted in human kidney [22, 23]. Glucose is actively transported from the lumen to the tubular cells by two transmembrane proteins: the high capacity-low affinity sodium-glucose transporter 2 (SGLT-2) and a high affinity-low capacity sodium-glucose transporter 1 (SGLT-1). The SGLT-2 is found in the S1 segment of proximal tubules and it has a low affinity (2.0 mM for glucose and 0.1 mM for sodium), but a high capacity for glucose transport with a 1:1 stoichiometry for sodium and glucose. Conversely, the SGLT-1, located in the S3 segment of the proximal tubules has a high affinity (0.4 mM for glucose and ~3.0 mM for sodium), but a low capacity for glucose transport with a 2:1 stoichiometry for sodium and glucose [15-17, 21]. SGLT-1 is also abundant in the enterocytes of the intestinal mucosa, where it is involved in glucose absorption and is apparently essential for the stimulation of the gastrointestinal insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1) secretion from the K- and L-cells, respectively in the distal intestine [24].

Filtration and tubular transport

Considering that nearly 180 liters of plasma are filtered daily by the kidneys and assuming the average plasma glucose is 100 mg/dl, the glucose load that crosses the glomeruli is estimated to be ~180 grams per day (Figure 2). Circulating glucose is neither protein-bound nor attached to macromolecules and thus, is freely filtered at the glomerulus. The ultrafiltrate carries glucose towards the luminal side of the S1 segment in the proximal tubules, where SGLT-2 is located. The active process of re-uptake of glucose is coupled with the transport of sodium cations and the

"Sodium-Glucose" complex is transferred to the cell. The SGLT transport activity across the luminal membrane is driven by an electrochemical gradient, which is created by the action of the ATPase-mediated sodium-potassium pump located in the baso-lateral membrane of the cells [22]. As glucose builds up inside the proximal tubular cells, a facilitated passive transport driven by a concentration gradient and mediated by GLUT-2 transporters, transfers intact glucose molecules from the cell to the surrounding renal interstitial fluid [21]. In addition to restoring glucose to the interstitial fluid and to the peripheral circulation, this sodium co-transport process also contributes to the maintenance of fluid and electrolyte balance by the kidney [15].

Under normal circumstances, the SGLT-2 co-transport system is responsible for the reabsorption of nearly 90% of all filtered glucose load. The reabsorption of the remaining 10% of the glucose load is dependent upon the activity of the SGLT-1 co-transporter and takes place downstream in the proximal tubules. The fact however that SGLT-2 is exclusively found in the proximal tubules of the kidney, as opposed to SGLT-1 or other transporters including the GLUT-2, makes it suitable for more specific renal pharmacologic interventions. As a result, the possibility of interfering with the activity of the SGLT-2 gained importance and became of considerable clinical significance.

Glucose production and utilization

Unlike the cells in the proximal nephron, those in the distal nephron are fully capable of glucose utilization. In physiologic conditions, the amount of glucose utilized in the distal segments of the nephron often equals the amount of glucose produced by renal gluconeogenesis in proximal tubules, and the arterial-renal vein blood glucose concentration difference is near zero [25-27]. This is not the case however in more prolonged fasting conditions and during hypoglycemia, when a net contribution of the kidney to systemic glucose appearance has been demonstrated [8, 9, 25, 26]. It has been postulated that renal glucose production exceeds utilization in patients with diabetes and thus, a net release of glucose by the kidney may contribute to hyperglycemia [27, 28], although this might reflect an increase in tubular glucose reabsorption [22, 29, 30]. Renal glucose utilization occurs exclusively in distal tubular cells and, in the absence of glucose-6-phospatase activity no glucose is released into the interstitial fluid [14]. It is not known whether glucose uptake in the distal nephron is mediated via an insulin-dependent mechanism or a facilitated transport [3, 6-9]. Renal glycogen accumulation is thought to provide for immediate local energy needs, when blood-borne glucose supply lags behind. Glucose oxidation can be partial (anaerobic glycolysis) with the release of lactate, or complete, a mitochondrial process that yields H_2O, CO_2 and ATP. Partial anaerobic glucose oxidation is more prevalent in hypoxic medullary renal conditions [11-14]. The amount of glucose utilized by the kidney is too small to affect the glucose load leaving the nephron into the renal vein.

Renal glucose kinetics

The fate of the glucose filtered, reabsorbed and excreted in the urine in normal and hyperglycemic conditions depends upon the glomerular filtration rate (GFR), the prevalent plasma concentration of glucose and the total transport capacity of the proximal tubules (Figure 3). There is a linear relationship between the filtered glucose load at the glomerulus and plasma glucose. Thus, the glucose appearance in the ultrafiltrate will be higher or lower, depending on the increase or decrease of plasma glucose concentration, respectively. To a lesser extent, the same is true for bi-directional changes in GFR. The proximal tubular reabsorption rate, on the other hand is linear within the normal glycemic range, but in conditions of extreme hyperglycemia this linear curve reaches a plateau. Once the maximum tubular reabsorption capacity of the kidney for glucose (*Tmax*) is attained, the transport process becomes saturated and glucose spills into the urine. It is worth mentioning that since the *Tmax* for glucose varies considerably among the nearly 2 million nephrons in both kidneys, the maximum transport capacity is actually a "splay" or a range of values estimated to be around the calculated *Tmax* [15, 21].

Maximum transport and renal threshold

The *Tmax* can be determined by artificially elevating plasma glucose levels in a stepwise

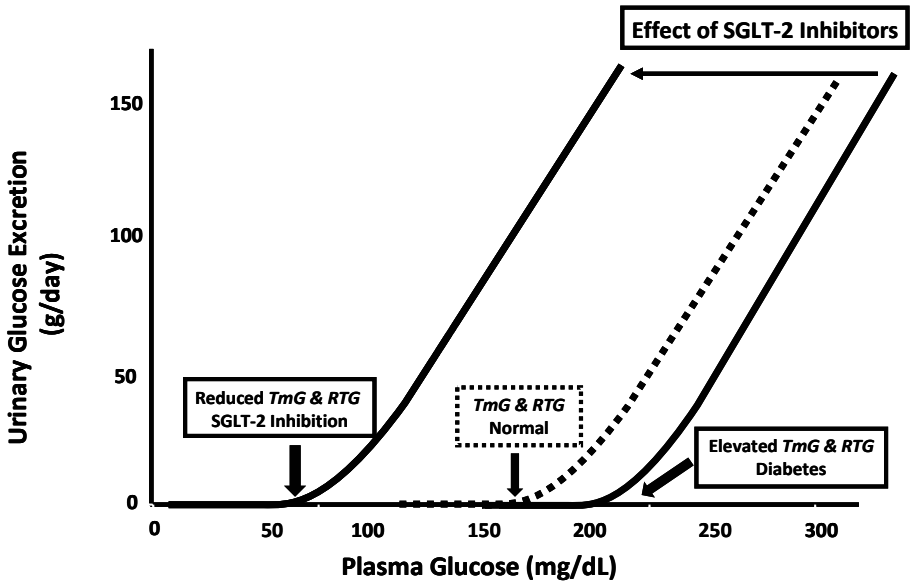

Figure 3. This graph depicts renal glucose kinetics in normal, diabetic conditions and following the action of SGLT-2 inhibitors. Urinary glucose excretion rate is expressed in grams/day in the vertical axis versus the renal threshold (*TmG*) and the plasma glucose concentration in mg/dl in the horizontal axis. In normal healthy individuals, the dotted line represents the rising urinary excretion rate once the RTG (~180 mg/dl) is achieved. The solid lines depict the urinary glucose excretion rates elevated in patients with diabetes (RTG ~220 mg/dl) and after the effect of SGLT-2 inhibitors, when the RTG value is substantially reduced (< 100 mg/dl).

fashion up to 400-500 mg/dl with simultaneous measurements of GFR and urinary glucose excretion, which allows the calculation of the rate of glucose reabsorption (i.e., glucose filtered minus glucose excreted) at given intervals. The "splay" derives from the rounding off of a range of values on the curves drawn for maximum glucose reabsorption and for glucose excretion rates. These two curves usually show a non-linear transition as the *Tmax* value is approached. In individuals with normal kidney function, the calculated maximum tubular glucose transport has been reported to vary between 350 mg/min in females and 450 mg/min in males, which corresponds to a mean arterial glucose concentration (RTG) of ~180 mg/dl. Hence, the normal renal threshold (*TmG*) is often achieved when plasma glucose concentration (RTG) is ~180 mg/dl [15, 21]. Once this value is exceeded, the SGLT transport capacity is surpassed and glycosuria ensues. *TmG* varies with changes in GFR such that during pregnancy or with a unilateral kidney, when GFR increases, glycosuria will occur at plasma glucose concentrations < 180 mg/dl (lower *TmG* and RTG). Conversely, when GFR decreases such as in chronic kidney disease, glycosuria is not seen until plasma glucose (RTG) exceeds 220 mg/dl [28]. Of additional interest, some healthy individuals inherit a genetic abnormality characterized by a defective SGLT transport system manifested by constant glycosuria within normal GFR and normoglycemia [31-33].

Maladaptation to hyperglycemia

The observation that the maximum tubular glucose reabsorption rate is markedly affected by chronic hyperglycemia has provided the rationale for a novel approach in the treatment of diabetes. Chronic exposure to hyperglycemia is accompanied by a 20-40% elevation in *TmG*, reflecting enhanced maximum glucose transport capacity and reabsorption [22, 29]. As a consequence, the glucose appearance in the urine occurs at plasma glucose concentrations above 180 mg/dl in patients with poorly-controlled diabetes. More recent data derived from *in vitro* studies using cultures of proximal renal tubular cells in urine samples of subjects with and without diabetes support the clinical observations [30]. Collectively, these results indicate that there is a "maladaptive"

response of the kidney to hyperglycemia in diabetes mellitus. By increasing glucose reabsorption rates, the kidney helps to maintain the abnormal status of hyperglycemia, which may in turn lead to further renal maladaptation.

Inhibitors of glucose transporters

Chemical development

The possibility that the diabetic kidney perpetuates hyperglycemia gave rise to the notion that agents capable of inhibiting renal glucose reabsorption might be useful in lowering blood glucose [10]. As originally envisioned, SGLT inhibitors would reduce the tubular capacity for glucose reabsorption (lower *TmG*) and promote glycosuria at lower plasma glucose levels (lower RTG). Although there was some excitement surrounding these earlier animal findings [10], the lack of selectivity of phlorizin, the associated adverse gastro-intestinal effects and the uncertainty regarding the consequences of the induced glycosuria dampened the initial enthusiasm for the clinical development of this agent. Additionally, the realization that phlorizin was quickly degraded by lactase-phlorizin hydrolase and that it was poorly absorbed in the intestines resulting in very low bioavailability halted any further investigation using nonspecific SGLT inhibitor in humans [34-36].

A search for SGLT-2 inhibitors that were more selective, more resistant to intestinal degradation and had higher plasma bioavailability was launched. Many agents with SGLT inhibitor properties were developed by chemically modifying the parent compound phlorizin ("second generation agents"). Initial experiments in cultured cell lines expressing human SGLT-1 and SGLT-2 transporters were conducted to determine the degree of selectivity of SGLT inhibitors using radio-ligand binding assays [37-39]. Following numerous attempts, a high degree of selective inhibition of SGLT-2 was shown for several compounds, though some did not progress to clinical development because they were vulnerable to degradation by the intestinal glucosidases [40]. A few compounds, such as dapagliflozin, empagliflozin, canagliflozin and ipragliflozin underwent further modifications and were shown to effectively circumvent the intestinal degradation step. These agents reached pre-clinical and clinical studies and are currently either approved or in the process of commercialization. Because these selective SGLT-2 inhibitors are minimally degraded in the intestinal tract, adequate bioavailability in the range of 33 to 80% was confirmed in humans [38-40].

Pharmacokinetics

The individual potency and selectivity of these agents were analyzed *in vitro* by measuring the uptake of the radio-labeled non-metabolizable glucose analogue [^{14}C]-alpha-methyl glucopyranoside (AMG) into cells over-expressing SGLT-1 and SGLT-2 [38], and compared to the non-selective parent compound phlorizin (Table 1). Even though the selectivity for SGLT-2 is nearly equivalent amongst all compounds studied, the pharmacological "window" of canagliflozin appears to be the closest to the non-selective SGLT inhibitor phlorizin. Consistent with these data, recent findings in humans [39] revealed that following oral ingestion of canagliflozin there was a decrease in the systemic appearance of glucose derived from the gut, documented during the initial 120 minutes post-meal. These have been interpreted as a result of a possible inhibitory effect on the intestinal glucose absorption of canagliflozin, the concentration of which is sufficiently high in the gut that it has some binding affinity to interact with the SGLT-1 transporters at the brush-border membrane of enterocytes. Thus, the activity of the sodium-glucose/galactose co-transport process may be reduced and a transient decline in the intestinal absorption of these sugars ensues. The selectivity for the renal SGLT-2 transporters is nevertheless preserved despite lower circulating drug levels attained after partial splanchnic clearance. Upon reaching the kidney specific SGLT-2 inhibitors bind avidly to SGLT-2 transporters in the luminal tubular membrane, whereas the binding affinity for the SGLT-1 transporter located downstream is severely diminished. Hence, whereas SGLT-2 inhibitory activity can be detected, there is no evidence that SGLT-1 in the kidney is also inhibited [37]. Following an insulin-independent decline in blood glucose, SGLT-2 inhibition is also accompanied by improvements in insulin

Table 1. Individual potency and selectivity of SGLT inhibitors based on AMG* percent uptake inhibition assay using vesicles over-expressing SGLT-1 and SGLT-2 transporters *in vitro*[@].

SGLT inhibitor	SGLT-1		SGLT-2	
	IC_{50}[†]	pI C_{50}[†]	IC_{50}	pI C_{50}
Empaglifozin	8300 nM	5.08 ± 0.03	3.1 nM	8.50 ± 0.02
Dapaglifozin	1400 nM	5.86 ± 0.07	1.2 nM	8.94 ± 0.06
Canagliflozin	710 nM	6.15 ± 0.06	2.7 nM	8.56 ± 0.02
Phlorizin[‡]	290 nM	6.54 ± 0.05	21 nM	7.67 ± 0.03

*AMG = [^{14}C]-alpha-methyl glucopyranoside.
[†]IC_{50} & pI C_{50} selectivity expressed as drug concentration (mean ± SEM) values required for 50% inhibition of AMG (or O-glucosides) uptake mediated via SGLT-1 and SGLT-2 transporters.
[‡]Phlorizin data obtained with *O-glucosides* uptake inhibition assay.
[@]Adapted from data in references 34 & 39.

sensitivity [41, 42]. This is important and represents an additional mechanism by which these agents can contribute to glycemic control in patients with type 2 diabetes. Of interest, recent data reported in a SGLT-2 knockout mouse model provide further evidence that this alternative approach (reducing renal threshold and promoting glycosuria) may enhance glycemic control, improve insulin sensitivity and preserve beta-cell function [42].

Clinical pharmacology

The maximum inhibitory effect achievable on renal glucose reabsorption with the use of selective blockade of the tubular SGTL-2 transporter activity in humans has been reported to be at 30-50% [43, 44]. The efficacy of these agents is somewhat limited, in part because of the competitive nature of the inhibitory binding process. There is also the possibility that very low levels of the active drug reach the tubular luminal membrane, the main site of the drug action. Finally, and perhaps most importantly, the extent to which a compensatory enhancement in the SGLT-1 co-transporter glucose reabsorption capacity (or even by a yet unidentified tubular glucose transport system) contributes to the low effectiveness of these agents has not been defined [15, 19]. Once plasma steady-state concentrations of the SGLT inhibitor are reached (4-5 days), the total amount of glucose excreted in the urine in non-diabetic subjects with normal GFR is around 50-80 grams per day. Recent clinical observational studies conducted in healthy non-diabetic and in diabetic subjects have actually indicated that the appearance of glycosuria can be detected within hours after the oral intake of a single dose of an SGLT-2 inhibitor [45, 46]. Of interest, glycosuria was present at plasma glucose values ranging anywhere from 40-120 mg/dl in these studies [41, 43], which derives from a partial blockade of the SGLT-2 co-transporter and reflects a substantial decrease in the renal threshold. The decrease in blood plasma glucose is accompanied by the loss of 300-320 calories daily. Preliminary data have demonstrated nevertheless that the decline in plasma glucose concentration with the use of SGLT inhibitors is attenuated by an increase in endogenous glucose production [39, 47]. Whether or not this compensatory increase in systemic glucose appearance is related to a simultaneous rise in plasma glucagon or to a decrease in circulating insulin or both remains unclear. It should be emphasized that these findings were documented in subjects who had received SGLT-2 inhibitors for a short period of time and had experienced a drop in plasma glucose levels, but without evidence of hypoglycemia. These intriguing observations have raised new questions regarding potential interactions between the kidney and liver in glucose regulation and counter-regulation, and will require confirmation.

Following oral administration, SGLT-2 inhibitors are rapidly absorbed with peak plasma concentrations (median T_{max}) occurring 1-2 hours post-dose. Plasma C_{max} and the area under the curve (AUC) increase in a dose-proportional manner with

apparent terminal half-life ($t_{1/2}$) varying from 10 to 13 hours [39, 41, 43, 48]. The active drug reaches a steady-state level within 4-5 days. The primary metabolic elimination of canagliflozin is via hepatic glucuronidation and some inactive metabolites are released into the peripheral circulation. There is minimal CYP3A4-mediated oxidative degradation and thus, clinically relevant effects of other drugs on canagliflozin pharmacokinetics via cytochrome P450 are unlikely to occur. Also, since the CYP450 enzyme system is not induced and is only minimally attenuated, only negligible changes in drugs utilizing the same hepatic metabolic processes have been reported. In contrast, a decrease in total exposure to active drug occurs when *UGT* (glucuronosyl transferase) inducers, such as rifampin, phenytoin and phenobarbital are co-administered. Also, plasma digoxin levels tend to increase and require closer monitoring when used in combination with canagliflozin. In normal healthy volunteers nearly 50% of the active drug is recovered in feces together with < 10% of some inactive metabolites, and < 1% is excreted intact in the urine [49]. Orally administered dapagliflozin is rapidly absorbed and peak plasma concentrations are reached within 2 hours. There is also dose-proportional systemic exposure to dapagliflozin over a wide range (0.1-500 mg) and ~78 % bioavailability. Its mean volume of distribution is 118 liters and metabolic degradation occurs predominantly in the liver with the formation of the inactive metabolite 3-O-glucuronide via the action of the uridine diphosphate-glucuronosyl-transferase-1A9. Dapagliflozin is not appreciably cleared by the kidney (< 2 % of dose is recovered in urine as parent drug), although the 3-O-glucuronide metabolite elimination occurs mainly via renal excretion and accounts for ~61 % of a single dose of dapagliflozin recovered in urine [50, http://www.medicines.org.uk/EMC/medicine/27188]. Similar pharmacokinetics has been described for empagliflozin [51, http://www.fda.gov]. There is currently very little information available for other SGLT-2 inhibitors in development.

Efficacy and safety of SGLT-2 inhibitors

Glucose lowering effect

The fact that selective inhibitors of SGLT-2 lower plasma glucose concentration in an insulin-independent manner, and thus with minimal risk of hypoglycemia, combined with the potential to induce simultaneous body weight loss has generated considerable clinical interest [46, 52-54]. Several SGLT-2 inhibitors are currently in development and some have already been approved by the Food and Drug Administration (FDA) for use in the treatment of patients with type 2 diabetes [49, 51; http://www.fda.gov]. Results from a few selected clinical trials are summarized in Table 2. Most studies have shown consistent and unequivocal improvements in glycemic control in a variety of diabetic patients with a decline in both fasting and postprandial plasma glucose. The range of the decrease in HbA_{1c}, from baseline and placebo-subtracted was reported between 0.24-1.16% and when SGLT-2 inhibitors were added to a comparator drug the decline in HbA_{1c} ranged between 0.37-1.98%. These results derive from a variety of clinical trials conducted during observation periods that varied from 16 weeks to 4 years (Table 2). It is reassuring to note that more recent additional data have confirmed these changes to be sustained with an acceptable safety profile for more prolonged time [55]. The degree of glycemic control attained with SGLT-2 inhibitors has been shown to be either comparable or superior to various anti-diabetic agents routinely recommended in standard practice [45, 46, 54, 55].

Diabetes treatment

The exact placement of SGLT-2 inhibitors in algorithms designed to guide management of diabetes remains undetermined and is somewhat controversial [56-58]. These drugs are effective in lowering blood glucose when used as monotherapy over a wide range of plasma glucose concentrations. This is not surprising since it has been established that the tubular glucose reabsorption threshold is already enhanced early, even in individuals with HbA_{1c} at 6.5% (46.5 mmol/mol) [41]. Because of limited clinical experience and considering the added cost, the “timing” for the introduction of this new class of drugs in the treatment of type 2 diabetes is likely to be later, rather than earlier. The possibility nevertheless of combining these agents with other anti-diabetic drugs, oral and injectables seems more reasonable. Given their unique mechanism of action, SGLT-2 inhibitors

Table 2. Reported clinical efficacy of various SGLT-2 inhibitors used in pivotal clinical trials in the treatment of type 2 diabetes[@].

SGLT-2 inhibitor	Placebo/ Comparator	Duration of Study	Baseline HbA_{1C}	Δ HbA_{1C} *	Δ Body* Weight (lbs)
Canagliflozin 100 mg 300 mg	vs. PLACEBO	26 weeks	~8.0% ~8.1%	- 0.91% - 1.16%	- 4.2 - 6.4
Canagliflozin 300 mg	vs. SITAGLIPTIN (+ MET & SU)	52 weeks	~8.1%	- 0.37%	- 5.2
Canagliflozin 100 mg 300 mg	vs. PLACEBO (+ Insulin & AHA)	26 weeks	~8.3%	- 0.69% - 0.73%	- 1.9 - 2.4
Dapaglifozin† 10 mg	vs. MET alone vs. COMBO	24 weeks	~8.1%	- 1.44% - 1.98%	- 3.2 - 4.8
Dapaglifozin 10 mg	vs. SU (+ MET)	52 weeks	~7.7%	- 0.18%	- 2.5
Dapaglifozin 10 mg	vs. SU (+ MET)	208 weeks (4 years)	~7.7%	- 0.30%	- 5.6
Empaglifozin‡ 5 mg 10 mg 25 mg	vs. PLACEBO	16 weeks	~7.9%	- 0.24% - 0.52% - 0.50%	- 3.0 - 5.2 - 4.8
Ipraglifozin‡ 50 mg	vs. PLACEBO	16 weeks	~8.3%	- 1.10%	- 5.2

* = Δ HbA_{1C} and Δ Body Weight = represent mean values of placebo-subtracted or comparator-subtracted values; degree variation not provided; values are statistically significant vs. placebo or comparator with $p < 0.05$, unless otherwise indicated.
†changes from baseline; ‡ = *p* values not available.
[@]Adapted from data in references 45, 46, 54 & 55.

may be best indicated in patients with poorly-controlled type 2 diabetes perhaps in those treated to exhaustion with oral drugs and in need of insulin replacement therapy. These suggested therapeutic options are not based on firm scientific evidence but represent one viewpoint. The ultimate decision as to when and how to best use SGLT-inhibitors during the management of diabetes will require additional data and long term experience.

Body weight loss

As anticipated, body weight loss occurred in almost all diabetic patients who received therapy with SGLT-2 inhibitors in various pivotal clinical trials [45, 46, 54, 55]. The usual amount of weight lost was in the range of 2-4 kilograms over 6 months, with only a few outliers. Interestingly, studies using other drugs that promote weight loss usually show substantial individual variability, whereas with SGLT-2 inhibitors a fixed amount of calorie loss is followed by a nearly equal absolute weight loss in just about everyone treated. A recent study indicated that the majority of the weight reduction is due to loss of fat mass, ~50% each in the abdominal and subcutaneous fat depots, with minimal changes in lean body mass [59]. Stabilization of the weight loss achieved after 6-12 months of therapy apparently persists for a longer period of time [59]. This is a major advantage and provides further reassurance to those who manage obese type 2 diabetic patients. Whether a far later compensatory increase in appetite and/or a change in energy expenditure will occur in response to the loss of calories in the urine should not be discarded. As a reminder,

these agents are not approved for the sole treatment of overweight and obese individuals, who do not have a diagnosis of type 2 diabetes.

Hypoglycemia and adverse events

The low incidence of hypoglycemia is a clinically relevant and important characteristic associated with the use of SGLT-2 inhibitors in the management of type 2 diabetes. This results from the fact that the mechanisms underlying the glucose-lowering effect of partial blockade of the tubular glucose reabsorption are insulin-independent and do not involve direct changes in insulin secretion. The rate of urinary glucose excretion is proportionate to the circulating plasma glucose concentration (i.e., glucose-dependent glucose excretion) and thus, glycosuria persists albeit reduced, with lower plasma glucose concentrations. The development of clinically significant hypoglycemia is prevented by the compensatory rise in endogenous glucose release, which also sustains the glycosuric effect [39, 41, 47]. In contrast, when SGLT-2 inhibitors are used in combination with insulin secretagogues (sulfonylureas, meglitinides) or together with insulin injections, the risk for hypoglycemia is magnified [51-53]. There is no currently approved indication for the co-administration of SGLT-2 inhibitors with insulin therapy in type 1 diabetes, just as there are no data on the safety and efficacy of these agents in pediatric patients under 18 years of age.

There is a noticeable increase in the incidence of urinary tract and genital infections in patients with type 2 diabetes treated with SGLT-2 inhibitors documented in most clinical trials [45, 46, 54, 55]. Nearly all urinary tract infections reported were limited to the lower tract and were present in ~5-13% of participants receiving SGLT-2 inhibitors vs. ~3-8% of those randomized to either placebo or a comparator drug. Similarly, genital infections developed in ~10-18% of patients taking SGLT-2 inhibitors, whereas those using placebo or a comparator drug had an incidence no higher than ~5%. These observations were derived primarily from studies that included a total of more than 20,000 patients with type 2 diabetes followed by at least 2 years of exposure to canagliflozin, dapagliflozin or empagliflozin [49-51]. Women, especially those with a positive past medical history of urinary tract and genital infections were more commonly affected. The vast majority of infections resolved with standard treatment, did not require hospital admissions and recurrences were infrequent. Actually, many participants who developed urinary and genital infections elected to continue with the treatment, especially if glycemic control and significant body weight loss were also apparent [51, http://www.fda.gov].

Some other relevant findings have been reported in patients with type 2 diabetes exposed to various SGLT-2 inhibitors in clinical trials (Table 3). A transient period of polyuria and urinary frequency with increased thirst has been described in 3-5% of all study subjects [45, 46, 54, 55]. Two-thirds of these individuals had symptoms of postural dizziness but no documented orthostatic hypotension. The majority recovered uneventfully, presumably because blood volume and fluid balance were appropriately corrected by alternate renal and other mechanisms. Of note, signs and symptoms of intra-vascular volume depletion were more common in elderly diabetic patients who were taking anti-hypertensive drugs and/or diuretics. Of notice, a 2-3% increase in hematocrit was seen in most subjects, possibly related to intravascular volume depletion. Despite the transient nature of these acute hemodynamic events, greater caution with special attention to this vulnerable population is recommended. Although the exact reason(s) for the slight and consistent decreases in systolic and diastolic blood pressure recorded after 6 months of therapy in nearly all diabetic patients are not entirely clear [45, 46, 54, 55], this is likely to be related to the “glucosuretic” effect of SGLT-2 inhibitors.

Rare cases of mild hyperkalemia following the administration of canagliflozin have been reported, primarily in patients with some degree of renal insufficiency [60]. Almost all diabetic patients who experienced serum potassium elevations were using potassium-sparing diuretics, angiotensin-converting enzyme inhibitors or angiotensin receptor blocking agents. We speculate that by further altering the tubular-glomerular feedback loop with the reduction of the sodium-glucose reabsorption in proximal tubules, these agents may exacerbate an underlying hyporeninemic-hypoaldosteronism state, commonly seen in type 2

Table 3. Commonly reported adverse events* of various SGLT-2 inhibitors used in pivotal clinical trials in the treatment of type 2 diabetes[@].

SGLT-2 inhibitor	Increased urinary frequency	Increased thirst	Hypotension	Hypoglycemia	Urinary tract infections	Genital infections
Canagliflozin 100 mg (n = 3,092)	174 (5.6%)	80 (2.6%)	20 (< 1.0%)	71 (2.3%)	171 (5.5%)	510 (16.3%)
300 mg (n = 3,085)	177 (5.7%)	70 (2.5%)	30 (< 1.0%)	104 (3.4%)	175 (5.7%)	545 (18.1%)
Dapaglifozin 5 mg (n = 1,145)	0	0	5 (0.4%)	25 (2.2%)	149 (13.0%)	155 (13.5%)
10 mg (n = 1,193)	1 (< 1.0%)	1 (< 1.0%)	5 (0.4%)	35 (3.0%)	131 (11.0%)	181 (15.1%)
Empaglifozin 10 mg (n = 495)	12 (2.5%)	1 (< 1.0%)	N/A	N/A	19 (4.0%)	49 (10.0%)
Ipraglifozin 50 mg (n = 62)	N/A	N/A	N/A	N/A	1 (4.0%)	2 (< 1.0%)

N/A = Data not available.
*Number (%) of events placebo or comparator-subtracted.
[@]Adapted from data in references 45, 46, 54 & 55.

diabetes [61]. No serious clinical consequences have yet been registered in association with hyperkalemia, which has not been described with other SGLT-2 inhibitors. Considering that SGLT-2 inhibitors should be given only to patients with eGFR above 45 ml/min/1.73 m^2 and at lower doses, the occurrence of hyperkalemia is expected to be a rare event. These agents are not indicated for the treatment of patients with eGFR below 45 ml/min/1.73 m^2 and in those with advanced end-stage renal disease undergoing renal dialysis simply because of inefficacy. In case of inadvertent drug overdose and intoxication, SGLT-2 inhibitors cannot be removed from the circulation efficiently by hemodialysis. Almost nothing is known about untoward effects associated with acute elevations and tissue accumulation of the native SGLT-2 inhibitor compounds and their metabolites [51]. In patients with mild-to-moderate hepatic insufficiency, there is no need for adjustment in dose, although the use of SGLT-2 inhibitors has never been tested in patients with severe hepatic insufficiency. Also, according to the FDA the use of SGLT-2 inhibitors is contra-indicated during pregnancy and in lactating diabetic women, since newborn animals exposed to this agent exhibit a multitude of kidney and urogenital malformations [51, http://www.fda.gov].

Results from one large pivotal study presented earlier to the FDA revealed 9 cases of bladder cancer out of 5,501 patients (0.16%) in association with the use of dapagliflozin, as opposed to only one out of 3,184 (0.03%) treated with placebo/ comparator [50, 51]. Careful analyses of the data showed that all bladder cancers occurred in males and that 7 of all 10 patients with the diagnosis had hematuria prior to the initiation of the study treatment. Furthermore, eight patients with bladder cancer were current or former smokers, five of them were diagnosed at < 6 months from the start of dapagliflozin therapy, and none were diagnosed with a treatment period longer than 24 months. Similar data regarding the dapagliflozin association with breast cancer were examined but it was concluded that there were too few events to establish causality. Upon further review of additional data the FDA granted approval for the use of dapagliflozin, contingent upon post-marketing studies, including one for close vigilance of bladder cancer, and restricted its use in diabetic patients with known bladder cancer [50]. Although it is reassuring to know that SGLT-2 transporter proteins are not expressed either in human bladder or in breast tissue [33], the chronic accumulation of large amounts of glucose in the bladder urine over time cannot be

entirely ruled out as a putative carcinogenic factor. On the other hand, there are no reports of increased incidence of malignancy associated with the use of either canagliflozin or empagliflozin in patients with type 2 diabetes [51, http://www.fda.gov].

Currently, there is no clinical evidence that the use of SGLT-2 inhibitors in patients with type 2 diabetes is associated with improved cardiovascular (CV) outcomes. There are several ongoing clinical trials, such as CANVAS (canagliflozin), DECLARE-TIMI 58 (dapagliflozin) and EMPA-REG OUTCOME (empagliflozin), designed specifically to examine the effects of SGLT-2 inhibitors on CV morbidity and mortality. Based on the available data we assume that these drugs are CV safe as they have not so far been associated with increases in major CV events [49-51]. Similarly, the long-term adverse effects of SGLT-2 inhibitors on glomerular filtration and tubular functions, as well as on calcium, mineral and bone metabolism are unpredictable. We can take some comfort on the knowledge that individuals with "familial renal glucosuria" are essentially disease-free and live near-normal lives [31-33]. Homozygous individuals tend to have glycosuria that varies from 15 up to 200 g/day, whereas pure heterozygous family members have either mild glucosuria or none at all [33, 34]. Despite the fact that this condition is characterized by persistent urinary glucose excretion, even within the normal range of plasma glucose concentration, these individuals generally maintain normal body weight. Functional assessment and histological analyses have yielded no evidence of renal glomerular dysfunction or tubular abnormalities. Hypoglycemia is uncommon in these subjects and the incidence of diabetes mellitus, chronic renal insufficiency and urinary tract infections is comparable to the general population [33, 34]. Little information on calcium, mineral metabolism and on bone health is available. The diagnosis of "familial renal glycosuria" nonetheless should be distinguished from other complex tubular disorders that are associated with glycosuria and some morbidity. Whether in type 2 diabetes the chronic use of SGLT-2 inhibitors will follow a benign course of events remains unknown.

SUMMARY AND CONCLUSIONS

In summary, the kidney plays an important role in glucose homeostasis, contributes to glucose regulation and counter-regulation, and by reabsorbing the filtered glucose also helps to preserve the energy balance. These remarkable functions are achieved by an active proximal tubular transport system that promotes complete glucose reabsorption together with glucose production that often matches renal glucose utilization. Hyperglycemia is associated with an increase in renal threshold (*TmG*) and glycosuria occurs at higher than normal plasma glucose concentrations (elevated RTG). As a consequence, specific inhibition of the large capacity renal sodium-glucose co-transporter 2 (SGLT-2) has emerged as a potential pharmacological intervention in diabetes which, by decreasing tubular glucose reabsorption, induces glycosuria and reduces blood glucose. Continuous loss of calories in the urine is accompanied by a sustained decrease in body weight/fat in nearly all obese type 2 diabetic patients. The occurrence of urinary tract infections and genital mycosis requires close monitoring. Also, the development of transient mild polyuria in a small percentage of patients with occasional hypotension, particularly in elderly diabetic patients, is of concern. Canagliflozin and dapagliflozin can be used safely in individuals with mild renal impairment, but not in those with eGFR < 60 (dapagliflozin) or < 45 ml/min/1.73 m^2 (canagliflozin, 100 mg). Whether there is long-term damage to the kidney, calcium and bone mineral metabolism is unpredictable and CV benefits are yet to be demonstrated with the use of these novel agents. Some agents are now approved in the US for the treatment of type 2 diabetes, as either monotherapy or in combination with other anti-diabetic medications. As long as patients can tolerate them, if used with caution and in the right patient, SGLT-2 inhibitors provide a unique insulin-independent therapeutic option in the management of type 2 diabetes patients.

ACKNOWLEDGMENTS

E.C wrote the manuscript, researched the data, takes responsibility for the content and is the guarantor of this article. The author wishes to thank the secretarial assistance of Lorrie Albarado and the expert review and contributions made by Ralph DeFronzo, MD, Professor of Medicine at the University of Texas Health Science Center at San Antonio.

CONFLICT OF INTEREST STATEMENT

In the past year, the author has received honoraria as a member of the Speaker Bureau for Bristol-Meyer Squib, Astra-Zeneca, Janssen Pharmaceuticals and Boheringer-Ingelheim.

REFERENCES

1. Krebs, H. A., Hems, R. A. and Gascoyne, T. 1963, Acta Biol. Med. Ger., 11, 607.
2. Cahill, G. F. Jr. 1970, New Engl. J. Med., 282, 668.
3. Cersosimo, E., Judd, R. and Miles, J. M. 1994, J. Clin. Invest., 93, 2584.
4. Cersosimo, E., Ajmal, M., Naukam, R. J., Molina, P. E. and Abumrad, N. N. 1997, Am. J. Physiol. (Endocrinol. Metab.), 272(35), E756.
5. Cersosimo, E., Molina, P. E. and Abumrad, N. N. 1998, Diabetes, 46, 643.
6. Strumvoll, M., Chintalapudi, U., Perriello, G., Welle, S., Gutierrez, O. and Gerich, J. E. 1995, J. Clin. Invest., 96, 2528.
7. Cersosimo, E., Garlick, P. and Ferretti, J. 1999, Am. J. Physiol. (Endocrinol. Metab.), 276(39), E78.
8. Ekberg, K., Landau, B. R., Wajngot, A., Chandramouli, V., Efendic, S., Brunengraber, H. and Wahren, J. 1999, Diabetes, 48, 292.
9. Cersosimo, E., Garlick, P. and Ferretti, J. 2001, Diabetes, 50, 2087.
10. Rosetti, L., Smith, D., Shulman, G. I., Papachristou, D. and DeFronzo, R. A. 1987, J. Clin. Invest., 80, 1037.
11. Wirthensohn, G. and Guden, W. 1986, Physiol. Rev., 66(2), 469.
12. Weiderman, M. J. and Krebs, H. A. 1969, Biochem J., 112, 149.
13. Klein, K. L., Wang, M. S., Torikai, W., Davidson, D. and Kenokawa, K. 1981, Kidney Intern., 20, 29.
14. Guder, W. and Ross, B. D. 1984, Kidney Int., 26, 101.
15. Wright, E. M. 2001, Am. J. Physiol. (Renal Physiol.), 280, F10.
16. Aronson, P. S. and Sacktor, B. 1975, J. Biol. Chem., 250, 6032.
17. Turner, R. J. and Moran, A. 1982, Am. J. Physiol. (Renal Physiology), 242, F406.
18. Hediger, M. A., Coady, M. J., Ikeda, T. S. and Wright, E. M. 1987, Nature, 330, 379.
19. Sacktor, B. 1989, Kidney Int., 36, 342.
20. Lee, W. S., Kanai, Y., Wells, R. G. and Hediger, M. A. 1994, J. Biol. Chem., 269, 12032.
21. Hediger, M. A. and Rhoads, D. B. 1994, Physiol. Rev., 74, 993.
22. Mogensen, C. E. 1971, Scand. J. Clin. Lab. Invest., 28, 101.
23. Kanai, Y., Lee, W. S., You, G., Brown, D. and Hediger, M. A. 1994. J. Clin. Invest., 93, 397.
24. Gorboulev, V., Schurmann, A., Vallon, V., Kipp, H., Jaschke, A., Klessen, D., Friedrich, A., Scherneck, S., Rieg, T., Cunard, R., Vehyl-Wichmann, M., Srinivasan, A., Balen, A., Breljak, D., Rexhepaj, R., Parker, H. E., Gribble, F., Reimann, F., Lang, F., Wiese, S., Sabolic, I., Sendtner, M. and Koepsell, H. 2012, Diabetes, 61, 187.
25. Cahill, G. F. Jr., Herrera, M. G. and Morgan, A. P. 1966, J. Clin. Invest., 45, 1751.
26. Owen, O. E., Felig, P., Morgan, A. P., Wahren, J. and Cahill, G. F. Jr. 1969, J. Clin. Invest., 45, 574.
27. Eid, A., Bodin, S. and Ferrier, B. 2006, J. Am. Soc. Nephrol., 17, 398.
28. Mather, A. and Pollock, C. 2011, Kidney Int., 79(Suppl. 120), S1.
29. Farber, S. J., Berger, E. Y. and Earle, D. P. 1951, J. Clin. Invest., 30, 125.
30. Rahmoune, H., Thompson, P. W., Ward, J. M., Smith, C. D., Hong, G. and Brown, J. 2005, Diabetes, 54, 3427.
31. Santer, R., Kinner, M., Lassen, C. L., Schneppenheim, R., Eggert, P., Bald, M., Brodhel, J., Daschner, M., Ehrich, J. H., Kemper, M., Volti, S. L., Neuhaus, T., Skovby, F., Swift, P. G., Schaub, J. and Klaerke, D. 2003, J. Am. Soc. Nephrol., 14, 2873.
32. Chen, J., Williams, S. and Feder, J. N. 2010, Diabetes Therapy, 1(2), 57.
33. Santer, R. and Calado, J. 2010, Clin. J. Am. Soc. Nephrol., 5(1), 133.
34. Ehrenkranz, J. R., Lewis, N. G., Kahn, C. R. and Roth, J. 2005, Diabetes Metab. Res. Rev., 21(1), 331.

35. Oku, A., Ueta, K. and Arakawa, K. 1999, Diabetes, 48, 1794.
36. Isaji, M. 2007, Current Opinion in Investigational Drugs, 8(4), 285.
37. Tsujhara, K. 1999, J. Med. Chem., 42(26), 5311-24.
38. Grempler, R., Thomas, L., Eckhard, M., Himmelsbach, F., Sauer, A., Sharp, D. E., Bakker, R. A., Mark, M., Klein, T. and Eickelmann, P. 2012, Diabetes, Obesity and Metabolism, 14, 83.
39. Polidori, D., Sha, S., Mudaliar, S., Ciaraldi, T., Ghosh, A., Vaccaro, N., Farrell, K., Rothenberg, P. and Henry, R. 2013, Diabetes Care, 36, 2154.
40. Bailey, C. J. 2011, Trends in Pharmacol. Sci., 32, 63.
41. DeFronzo, R. A., Hompesch, M., Kasichayanula, S., Liu, X., Hong, Y., Pfister, M., Morrow, L. A., Leslie, B. R., Boulton, D. W., Ching, A., LaCreta, F. P. and Griffen, S. C. 2013, Diabetes Care, 36, 3169.
42. Michael, J. J., Hui-Young, L., Andreas, L. B., Francois, R. J., David, W. F., Rebecca L. P., Xiaoxian, Z., Gilbert, W. M., Varman, T. S., Jean, M. W., Shulman, G. I. and Kibbey, R. G. 2011, Diabetes, 60, 890.
43. Komoroski, B., Vachharanji, N. and Boulton, D. 2009, Clin. Pharmacol. Ther., 85, 520.
44. Liu, J. J., Lee, T. W. and DeFronzo, R. A. 2012, Diabetes, 61, 2199.
45. Bailey, C. J., Gross, J. L., Pieters, A., Bastien, A. and List, J. F. 2010, Lancet., 375, 2223.
46. Nauck, M. A., Del Prato, S., Meier, J. J., Duran-Garcia, S., Rohwedder, K., Elze, M. and Parikh, S. 2011, Diabetes Care, 34, 2015.
47. Meovci, A., Solis-Herrera, C., Daniele, G., Eldor, R., Vanessa, T. F., Tripathy, D., Xiong, J., Perez, Z., Norton, L., Abdul-Ghani, M. A. and DeFronzo, R. A. 2014, J. Clin. Invest., 124(2), 509.
48. Tahrani, A. A., Barnett, A. H. and Bailey, C. J. 2013, Lancet Diabetes Endocrinol., 1, 140.
49. Food and Drug Administration Endocrinologic and Metabolic Drugs Advisory Committee. January 10th, 2013, Canagliflozin as an adjunctive treatment to diet and exercise alone or co-administered with other anti-hyperglycemic agents to improve glycemic control in adults with type 2 diabetes mellitus. JNJ-28431754 (Canagliflozin). NDA 204042.
50. Dapagliflozin (Forxiga), November 2012, Summary of product characteristics, Bristol-Meyers Squibb, Uxbridge UK.
51. Food and Drug Administration Advisory Committee (July 19, 2011; March 21, 2013 & December 12th, 2013 reports).
52. Wilding, J. P., Norwood, P., Tjoen, C., Bastien, A., List, J. F. and Fiedorek, F. T. 2009, Diabetes Care, 32, 1656.
53. Kipnes, M. S. 2011, Clin. Invest., 1, 145.
54. Rosenstock, J., Aggarwal, N., Polidori, D., Zhao, Y., Arbit, D., Usiskin, K., Capuano, G. and Canovatchel, W. 2012, Diabetes Care, 35, 1232.
55. Blonde, L. Abstract 1110-P. 2013, Presented at the 73rd Annual Meeting of the American Diabetes Association, Chicago, IL.
56. Standards of Medical Care in Diabetes, 2014 American Diabetes Association, Diabetes Care, Vol. 37, Suppl. S11.
57. American Diabetes Association and the European Association for the Study of Diabetes, 2013, Curr. Med. Res. Opin., 29(7), 793.
58. Handelsman, Y. and AACE Task Force for Developing Diabetes Coimprehensive Care Plan. 2011, Endocrine Practice, 17(Suppl. 2), 1-53.
59. Bolinder, J., Ljunggren, O., Kullberg, J., Johansson, L., Wilding, J., Langkilde, A. M., Sugg, J. and Parikh, S. 2012, J. Clin. Endocrinol. Metabol., 97, 1020.
60. Woo, V. Abstract 73-LB, 2013, Presented at the 73rd Annual Meeting of the American Diabetes Association, Chicago, IL.
61. Knochel, J. P. 1979, Annual Review of Medicine, 30, 145.