ARTICLE

A Quantitative Systems Pharmacology Kidney Model of Diabetes Associated Renal Hyperfiltration and the Effects of SGLT Inhibitors

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The early stage of diabetes mellitus is characterized by increased glomerular filtration rate (GFR), known as hyperfiltration, which is believed to be one of the main causes leading to renal injury in diabetes. Sodium-glucose cotransporter 2 inhibitors (SGLT2i) have been shown to be able to reverse hyperfiltration in some patients. We developed a mechanistic computational model of the kidney that explains the interplay of hyperglycemia and hyperfiltration and integrates the pharmacokinetics/pharmacodynamics (PK/PD) of the SGLT2i dapagliflozin. Based on simulation results, we propose kidney growth as the necessary process for hyperfiltration progression. Further, the model indicates that renal SGLT1i could significantly improve hyperfiltration when added to SGTL2i. Integrated into a physiologically based PK/PD (PBPK/PD) Diabetes Platform, the model presents a powerful tool for aiding drug development, prediction of hyperfiltration risk, and allows the assessment of the outcomes of individualized treatments with SGLT1-inhibitors and SGLT2-inhibitors and their co-administration with insulin.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ GFR is regulated by the mechanism called the TGF. This mechanism is disturbed in the state of diabetes, leading to hyperfiltration in early stages, and to CKD in later stages of diabetes.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ This study presents a computational model describing the relationship between hyperglycemia and glomerular hyperfiltration. The model is used to investigate the causes of hyperfiltration development and the beneficial effects of SGLT inhibitors.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Although hyperglycemia leads to initial increase of GFR, kidney growth may be considered as the driving factor in development of hyperfiltration. SGLT inhibitors may prevent the increase of hyperglycemia-induced GFR and slows down the progression of hyperfiltration.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

☑ The developed model opens new opportunities for personalized predictions of hyperfiltration risk and SGLT inhibitor treatment outcomes. It allows personalized assessment of drug combination efficacy and may aid the development of new drugs by translating *in vitro* data into *in silico* predictions.

Diabetes mellitus (DM) is recognized as the leading cause of end-stage renal disease (ESRD), and 20–40% of patients with diabetes develop chronic kidney disease (CKD).[1] CKD is characterized by structural kidney damage (e.g., albuminuria as a marker) and impaired function, as reflected by a reduced glomerular filtration rate (GFR).[2] Although various mechanisms of CKD progression in diabetes have been proposed, the question of what triggers the onset of the disease has not been answered, yet.[3]

CKD in diabetes is preceded by hyperfiltration (i.e., abnormally high levels of total GFR).[4] The increase of total GFR is caused by higher single-nephron GFR (snGFR) rather than by increased nephron number, and is a consequence of the activation of the so-called renal functional reserve.[5] It is hypothesized that hyperfiltration leads to increased stress on nephrons and, ultimately, to glomerulosclerosis observed in ESRD. The hypothesis is supported by the observation that increased snGFR is associated with increased

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glomerulosclerosis and arteriosclerosis, obesity, and a family history of ESRD.[6]

Activation of the renal functional reserve and, by that, the hyperfiltration, can be triggered by different factors and (patho-)physiological states, such as high protein diet, pregnancy, DM, and others.[4,5] The key regulator of snGFR is a set of cells in the distal tubule located in direct proximity to the glomerulus called macula densa (MD). MD senses concentrations of sodium (Na^+), chloride (Cl^-), and potassium (K^+) ions in the filtrate. Increased concentrations of any of these ions invoke reduction of GFR, whereas reduced concentrations lead to an increase of GFR by vasodilation of the afferent arterioles.[7] This mechanism of GFR regulation by MD is known as the tubuloglomerular feedback (TGF).[4,8] Further regulation of GFR occurs via neural and hormonal pathways.[9]

Sodium is reabsorbed through various transporter proteins, including sodium-glucose cotransporter (SGLT)1 in the proximal straight tubule (PST) and SGLT2 in the proximal convoluted tubule (PCT). It is, therefore, not surprising that SGLT2 inhibitors (SGLT2i), a class of oral antidiabetic medications, receive close attention regarding their renoprotective effects.[10] SGLT2i reduce hyperglycemia by blocking glucose reabsorption in the proximal tubule (PT) and by inducing urinary glucose excretion (UGE). As a positive side effect, it has been observed that SGLT2i may counteract hyperfiltration[10] and slow down the progression of CKD.[11,12] Years before SGLT2i were introduced, Vallon *et al.*[3] showed that phlorizin, a nonspecific SGLT inhibitor, leads to decreased snGFR. Later, GFR reduction by SGLT2i has been shown in patients with type 1 and type 2 DM as well as healthy subjects.[7,13,14] In addition, acute GFR lowering effects of the SGLT2i dapagliflozin and empagliflozin were observed during a stepped hyperglycemic clamp (SHC).[13,15]

The focus on therapeutic intervention with SGLT2 rather than SGLT1, or unspecific, inhibitors is explained by the exclusive expression of SGLT2 in the renal tubule, whereas SGLT1 is the main transporter responsible for the uptake of orally ingested glucose from intestinal lumen. Inhibition of intestinal SGLT1 causes glucose malabsorption and gastrointestinal side effects.[16] Nevertheless, SGLT1 as a therapeutic target in diabetes holds great potential, and treatment opportunities are being investigated.[17]

Our understanding of the relationship between diabetes and diabetic kidney disease associated with hyperfiltration, and the progression of the diseases is hindered by the complexity of the processes involved. Early diabetes is characterized by increased kidney volume due to hyperplasia and hypertrophy of the PT cells,[18] and overexpression of SGLT2.[14] The regulation of glucose homeostasis and renal function involves a number of feedback loops and interactions, thus, the distinction between the cause and the consequence is often not trivial. Computational systems pharmacology models give the opportunity to study the complex regulatory networks and assess disease progression or treatments with *in silico* simulations. Our objectives, therefore, are (i) to develop a model of renal hyperfiltration to extend the open source physiologically based pharmacokinetics (PBPK) and pharmacodynamics (PD) Quantitative Systems Pharmacology (QSP) Diabetes Platform,[19,20] (ii) to extend and benchmark it with a model of pharmacological intervention with SGLT2i, and (iii) to investigate the potential efficacy of renal SGLT1i.

METHODS

Software

The models were developed with PK-Sim and MoBi as part of the Open Systems Pharmacology Suite (OSPS) version 7.1.[21] Exploratory simulations were performed and figures were created in R[22] with the OSPS Toolbox for R.

Modeling workflow

The presented open source differential equation-based QSP model is a combination of multiple PBPK models, linked by a network of PD effects and mechanistic processes. The PBPK concept relies on *a priori* knowledge of (human) physiology and the specific properties of compounds to describe their kinetics. The organism is represented by organs connected by blood and lymph flows and further divided into the relevant subcompartments. This division allows modeling the dependency of PD effects on the pharmacokinetics (PKs) of the compound at the physiological site of action. An overview of PBPK modeling is given in Kuepfer *et al.*[23]

The developed model is based on the standard PBPK model implemented in PK-Sim applying the distribution model for proteins.[24] The spatial structure of the kidney was extended for the areas of relevance for the current objectives, and the processes describing glomerular filtration and tubular reabsorption of glucose and Na^+ and the TGF were included. A PBPK model of dapagliflozin was developed as a representative for the class of SGLT2i medication. The extended kidney and dapagliflozin PBPK models were coupled and integrated into the PBPK QSP Diabetes Platform.[19,20]

Parameters of the dapagliflozin PK model were identified by fitting the model to reported plasma time-concentration profiles of dapagliflozin.[25–28] Parameters of the kidney model and the PD of dapagliflozin were estimated by fitting the model to UGE and GFR data from the SHC performed by Defronzo *et al.*[15] UGE data and plasma glucose concentrations during the clamp are reported by Lu *et al.*[29] Although measurements of GFR were performed at each clamp step, only mean GFR values are reported in the original publication.[15] Dose-dependency of dapagliflozin PD was fitted to UGE data from the single ascending dose study reported by Komoroski *et al.*,[27] simulated with the extended QSP Diabetes Platform. The datasets are listed in **Table S1**. For detailed descriptions of the experimental protocols, the reader should refer to the original publications.

Evaluation of the PBPK model was performed by visual predictive check plots (i.e., comparison of simulated and reported dapagliflozin time-concentration profiles), and representative goodness-of-fit plots. Simulated values within the twofold range (0.5–2-fold) of the observed ones were considered to be well-described.

The mechanisms of hyperfiltration development were assessed by simulating the SHC[15] with variation of selected parameters for ± 20% of the reference value as a local sensitivity analysis.

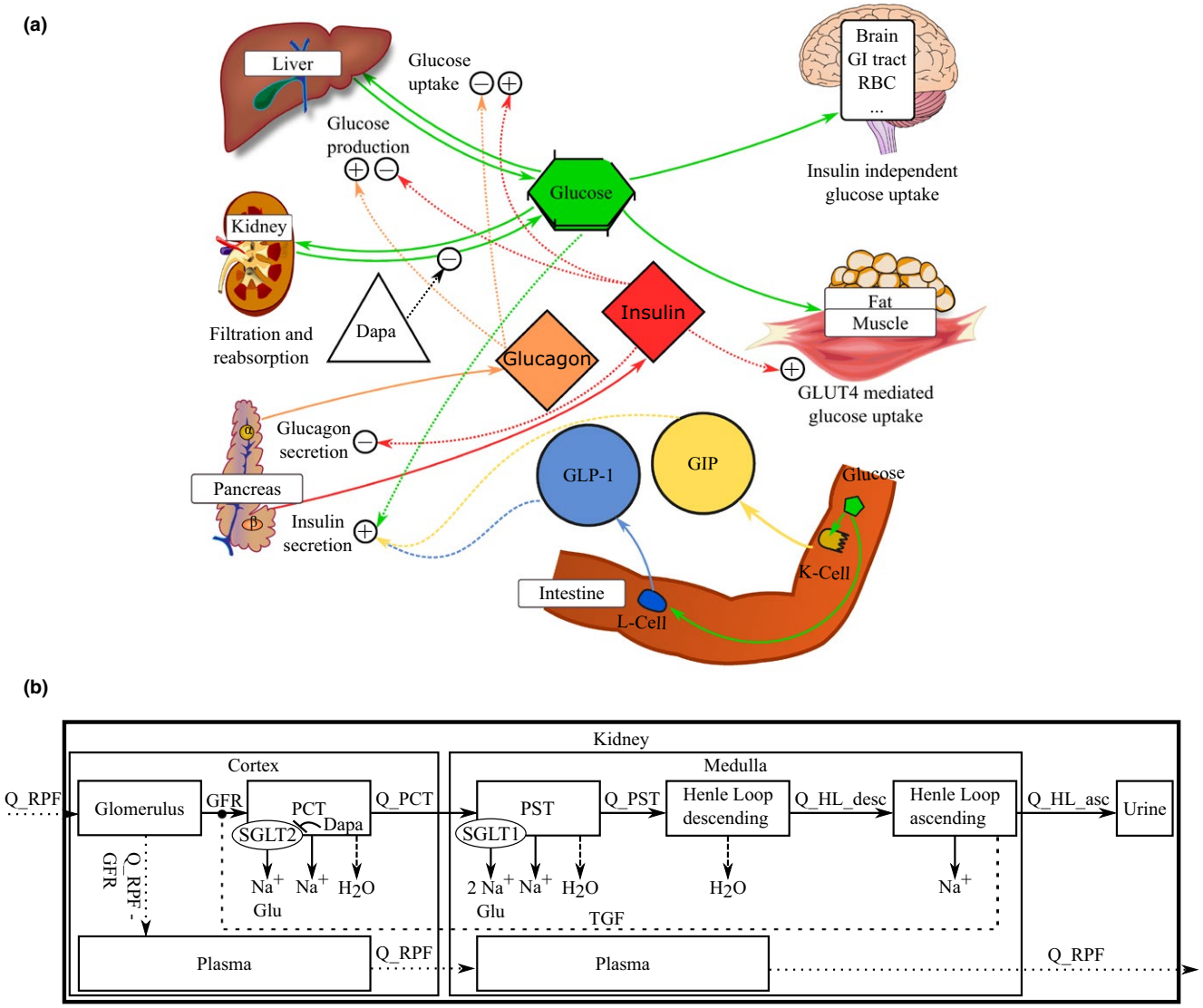

Figure 1 Structure of the model. The extended kidney and dapagliflozin PBPK models are integrated into the whole-body model of glucose homeostasis.[19,20] (**a**) Schematic representation of the implemented processes on the whole-body level. Solid lines represent the most important transport processes in the relevant organs; dashed lines represent the pharmacodynamic (PD) effects. Not all implemented PD effects are shown. (**b**) Detailed structure of the kidney model. Dapa, dapagliflozin; GFR, glomerular filtration rate; GI, gastrointestinal; Glu, glucose; HL, Henle Loop; PCT, proximal convoluted tubule; PST, proximal straight tubule; Q_HL_asc, filtrate flow rate from the ascending HL; Q_HL_desc, filtrate flow rate from the descending HL; Q_PCT, filtrate flow rate from PCT; Q_PST, filtrate flow rate from PST; Q_RPF, renal plasma flow; RBC, red blood cell; TGF, tubuloglomerular feedback.

To explore the renal effects of a potential SGLT1i or an unspecific SGLTi, simulations of the SHC clamp with fixed inhibition of either SGLT1 or SGLT2, or combined inhibition, were performed. A potential therapeutic effect on the whole-body level was examined by simulating the Diabetes Platform with normal or impaired insulin sensitivity and inhibition of renal SGLT.

RESULTS

The kidney and SGLT2i dapagliflozin models

The existing open source Diabetes Platform[20] has a rather limited representation of the kidney and does not offer structures or processes necessary for consideration of hyperfiltration and SGLTi medication. We, therefore, developed a new kidney model and a PBPK model of the SGLT2i dapagliflozin and integrated them into the Diabetes Platform. The final model incorporates PBPK submodels of glucose, insulin, glucagon, and dapagliflozin, and the regulation of glucose homeostasis at the whole-body level (**Figure 1a**).

The structure of the kidney model is outlined in **Figure 1b**, and a detailed description is provided in **Data S1**. The parameters of the model and their values are listed in **Table 1**. The organ is divided into the zones cortex and medulla, each having the standard PBPK model subcompartments: blood plasma and cells, interstitial, intracellular, and endosomal space. The nephron includes the compartments glomerulus and PCT in the cortex, and PST and the descending and ascending limbs of the Henle loop (HL) in the medulla. The

Table 1 Parameters of the kidney model

Parameter	Unit	Reference value	Value in simulation	Description	Reference
V_{cortex}	L	63% of kidney	0.28	Volume of cortex	37
$V_{medulla}$	L		0.16	Volume of medulla	
V_{glom}	L	3% of kidney	0.01	Volume of glomerulus	38
V_{PCT}	L	30% of cortex	0.08	Volume of PCT	39
V_{PST}	L	50% of PCT	0.04	Volume of PST	
V_{HL_desc}	L	50% of PCT + PST	0.06	Volume of HL_desc	40
V_{HL_asc}	L	25% of PCT + PST	0.03	Volume of HL_asc	40
C_{AB}^{Na}	mmol/L	141	141	Na^+ concentration in AB plasma	41
k_{cat}^{SGLT2a}	1/minute		17,896	Rate constant of SGLT2 mediated glucose transport	
V_{max}^{SGLT1a}	µmol/minute		322	Maximal rate of SGLT1 mediated glucose transport	
$K_m^{SGLT2,G}$	µmol/L	2,000	2,000	Glucose affinity to SGLT2	36
$K_m^{SGLT1,G}$	µmol/L	400	400	Glucose affinity to SGLT1	36
$k_{reabs,PCT}^{Na\ a}$	1/minute	0.56[b]	0.71	Rate constant of unspecific Na^+ reabsorption	42
$k_{reabs,PST}^{Na\ a}$	1/minute	0.56[b]	0.8	Rate constant of unspecific Na^+ reabsorption	42
k_{reabs,HL_asc}^{Na}	1/minute	1.34[b]	1.34	Rate constant of unspecific Na^+ reabsorption	42
R_{PCT}	$\frac{L^2}{\mu mol \times min}$	1e-4	1e-4	Water conductivity	
R_{PST}	$\frac{L^2}{\mu mol \times min}$	1e-4	1e-4	Water conductivity	
R_{HL_desc}	$\frac{L^2}{\mu mol \times min}$	8.75e-8[c]	8.75e-8	Water conductivity	9
$V_{max}^{TGF\_inc}$			1	Maximal rate of TGF increase	
α_TGF_inc			6	Exponent factor of TGF increase	
$K_m^{TGF\_inca}$	µmol/L		43,593	Target Na^+ concentration in HL_asc	
k_{dec}^{TGF}	1/minute		0.5	Rate constant of TGF decrease	
$k_{off}^{dapa,SGLT2a}$	1/minute	$\ll$0.14	0.04	Rate constant of dapa dissociation from SGLT2	43
$K_d^{dapa,SGLT2a}$	µmol/L	6e-3	1.57e-3	Affinity of dapa to SGLT2	43

All values refer to a standard 30-year-old male individual with kidney volume of 0.44 L.
AB, arterial blood; dapa, dapaglozin; HL_asc, ascending limb of Henle loop; HL_desc, descending limb of Henle loop; PCT, proximal convoluted tubule; PST, proximal straight tubule; SGLT, sodium-glucose cotransporter; TGF, tubuloglomerular feedback.
[a]Parameter values identified by fitting the model to urinary glucose excretion and glomerular filtration rate (GFR) data.[15,29] [b]Calculated based on the fractional extraction data. [c]Calculated based on the reabsorption of 15% of GFR.

distal tubule and collecting duct are not explicitly modeled. Glucose, Na^+, and water are filtered from plasma at the glomerulus and reabsorbed along the nephron. The model includes explicit transport of glucose and Na^+ by SGLT2 in PCT and SGLT1 in PST. The TGF regulates GFR based on the concentration of Na^+ in the ascending limb of the HL, which serves as a representative for the concentration at MD.

The PBPK model of dapagliflozin as a representative for the class of SGLT2i includes metabolization by the enzymes CYP3A4 and UGT1A9.[30] Distribution in tissues is calculated by the OSPS, including P-glycoprotein-mediated transport as an active process.[30] The drug is filtered in the glomeruli and reabsorbed in the PT of the kidney. Inhibition of SGLT2 is modeled as reversible binding of dapagliflozin to the transporter in the PT. A detailed description of the PBPK model of dapagliflozin is provided in **Data S1**; the parameters are listed in **Table S2**. The performance of the dapagliflozin model is good, as illustrated in **Figure S1**. Of the 369 compared points, only 1.9% (7 points) were outside of the twofold deviation range. Detailed statistics on the deviations for each dataset are provided in **Table S3**.

Simulations of the SHC

The SHC performed by Defronzo *et al.*[15] was simulated to assess the performance of the model and analyze the mechanisms of GFR regulation; the results are presented in **Figure 2**. In the simulation, 10 mg of dapagliflozin was administered 1 hour before clamp start, and SGLT2 remained inhibited between 97% at the beginning and 92% at the

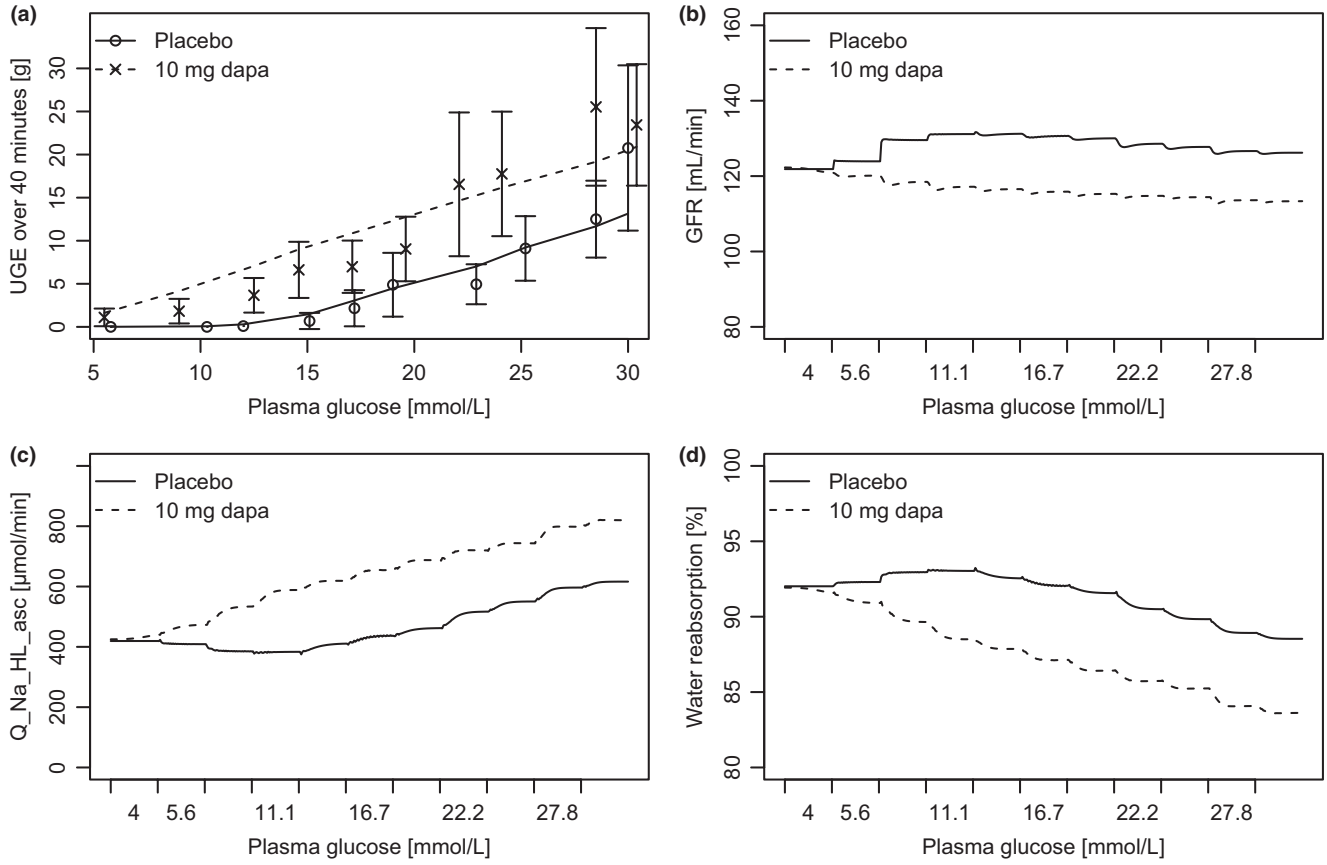

Figure 2 Simulations of the stepped hyperglycemic clamp (SHC). The SHC reported by Defronzo *et al.*[15] was simulated with (dashed lines) and without (solid lines) administration of 10 mg dapagliflozin. (**a**) Simulated (lines) urinary glucose excretion (UGE) over 40 minutes at rising plasma glucose concentration steps are compared to observed (symbols ± SD) data. (**b**) Simulated glomerular filtration rate (GFR). Mean GFR without and with dapagliflozin is 129 and 116 mL/minute, respectively. (**c**) Simulated Na^+ flow rate in the ascending limb of Henle Loop. (**d**) Simulated total rate of water reabsorption in % of GFR. The values of the *x*-axes in b–d show the target plasma glucose during each step; the measured and simulated concentrations were slightly different. Q_Na_HL_asc, Na^+ flow rate out of the ascending limb of Henle Loop.

end of the procedure. The model accurately reproduces observed UGE without administration of dapagliflozin for all but the highest glucose concentrations (**Figure 2a**, solid line, open circles). Simulated UGE with dapagliflozin administration is in good semiquantitative agreement with data but less accurate, being slightly overpredicted at lower and underpredicted at higher plasma glucose concentrations (dashed line, multiplication signs). The predicted correlation between UGE and plasma glucose after dapagliflozin administration is almost linear.

The simulated GFR in dependence of plasma glucose is presented in **Figure 2b**. Mean (as arithmetic mean of the values at the end of each clamp step) simulated value without dapagliflozin is 129 mL/minute (reported 128 ± 36 mL/minute) and is reduced by 13 mL/minute with administration of dapagliflozin (reported decrement of 17 ± 19 mL/minute).[15] Without SGLT2i, the GFR increases up to plasma glucose concentration of ~11.1 mmol/L, and decreases with further increasing glucose concentrations, but remains continuously elevated compared to the basal state of 4 mmol/L.

Changes in GFR ensure that Na^+ concentration in the ascending HL remains constant (simulated 43.1 mmol/L). Although SGLTs are not saturated (up to ~11 mmol/L plasma glucose), increased Na^+ reabsorption results in decreased Na^+ delivery to the ascending limb of HL. The consequence is the reduced Na^+ reabsorption in the ascending limb of HL, and, by that, reduced water reabsorption from the descending HL, as the latter is driven by the interstitial Na^+ concentration. With less water being reabsorbed, concentration of Na^+ reaching MD would decrease because of diluted filtrate. This is counteracted by increased GFR.

When plasma glucose concentration exceeds 11.1 mmol/L, SGLTs become saturated and glucose concentration in the filtrate rapidly increases. Being osmotically active, glucose causes retention of water in the filtrate and the fractional water reabsorption decreases, as can be seen in **Figure 2d**. This leads to higher filtrate flow rate and increased Na^+ flow in the ascending limb of HL (**Figure 2c**), inducing GFR inhibition through TGF.

Administration of dapagliflozin blocks the reabsorption of glucose and Na^+ in the PT, and the increased osmolality of the filtrate prevents the water from being reabsorbed. The flow of Na^+ in the descending loop of HL constantly

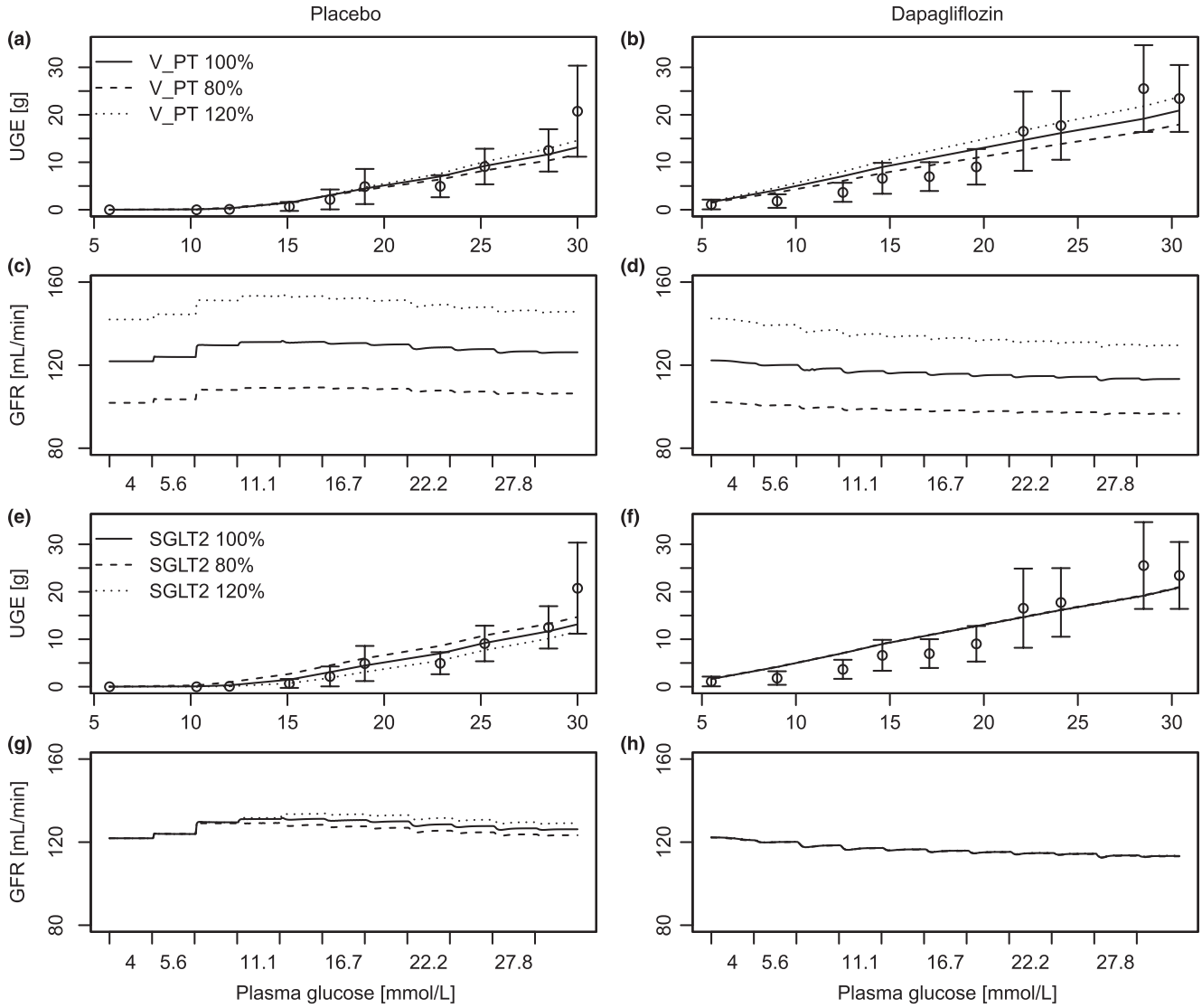

Figure 3 Impact of kidney growth and sodium-glucose cotransporter (SGLT)2 overexpression on glomerular filtration rate (GFR) and urinary glucose excretion (UGE). The stepped hyperglycemic clamp by Defronzo *et al.*[15] was simulated with selected parameters variated by ± 20% of the reference value. Simulated GFR and UGE without (left panels) and with (right panels) dapagliflozin administration are compared with simulations with reference parameter value (solid lines). (**a–d**) Variation of proximal tubular volume (V_PT) as sum of the proximal convoluted and straight tubule volumes. (**e–h**) Variation of SGLT2 expression. The values of the *x*-axes in **c, d, g, and h** show the target plasma glucose during each step; the measured and simulated concentrations were slightly different.

increases with increasing plasma glucose, invoking a negative feedback on GFR.

In silico studies of hyperfiltration development

Simulations were performed to investigate the possible mechanisms of hyperfiltration development based on the observed properties of the diabetic kidney. SHC was simulated with ± 20% variation of PT volume (observed change of PT length in diabetic rats was + 27%)[18] and SGLT2 expression, and UGE and GFR were compared to reference results in **Figure 3**.

Kidney growth in early diabetes is attributed to hyperplasia and hypertrophy of the PT cells, resulting in increased PT length and luminal volume.[18] Such morphological change was simulated by increasing the volume of PCT and PST. Although the implemented volume refers to the luminal space of the tubule, its change mimics an equal relative change of the cellular volume. The model predicts a strong effect of tubular volume (**Figure 3a–d**) on GFR irrespectively of dapagliflozin administration. Reducing tubular space by 20% lowers the mean GFR to 108 and 98 mL/minute without and with dapagliflozin administration, respectively (reference values 129 and 116 mL/minute). The decreased filtration rate results in a reduced amount of excreted glucose, with the reduction of UGE being more prominent under dapagliflozin treatment. The simulated 20% tubular growth results in markedly increased mean GFR of 149 and 133 mL/minute without and with dapagliflozin, respectively. UGE is slightly

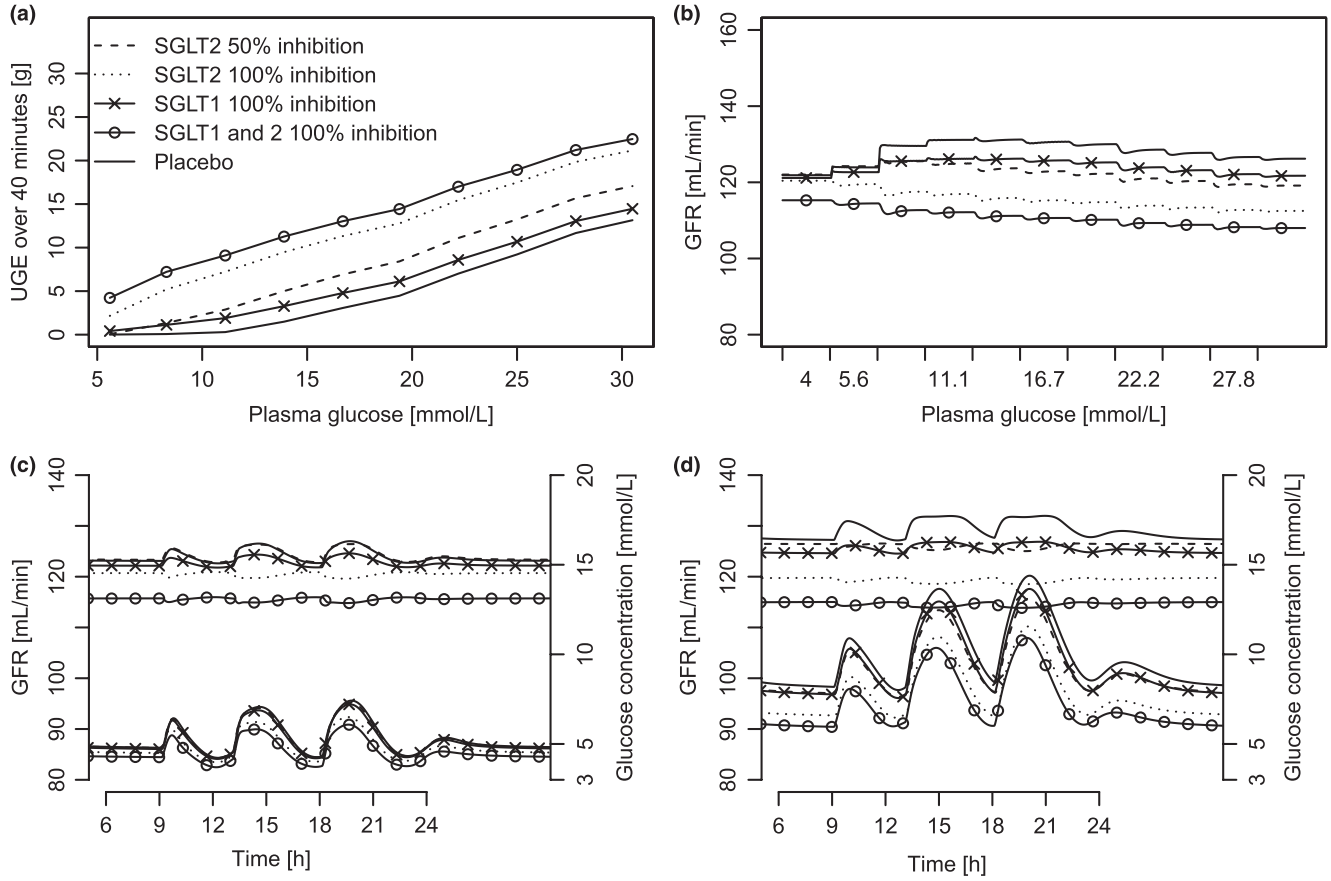

Figure 4 Simulations of static inhibition of sodium-glucose cotransporter (SGLT)2 and/or SGLT1. (**a**) Simulated urinary glucose excretion (UGE) over 40 minutes during the stepped hyperglycemic clamp (SHC). (**b**) Simulated glomerular filtration rate (GFR) during the SHC. (**c**) Glucose concentrations (right *y*-axis, bottom lines) and GFR (left *y*-axis, upper lines) at the 14th day of the Diabetes Platform simulation of a healthy individual. (**d**) Glucose concentrations (right *y*-axis, bottom lines) and GFR (left *y*-axis, upper lines) at the 14th day of the Diabetes Platform simulation of an individual with impaired insulin sensitivity. The values of the *x*-axis in **b** show the target plasma glucose during each step; the measured and simulated concentrations were slightly different.

increased, with absolute increment being greater at high glucose concentrations and more prominent with dapagliflozin administration.

In vitro experimental data show that isolated PT cells have higher levels of SGLT2 mRNA when incubated with high glucose.[14] The effect of SGLT2 expression was assessed by varying the expression level of the transporter (**Figure 3e–h**). No changes in the simulated results are observable with dapagliflozin administration. Without dapagliflozin, 20% reduction of transporter expression results in mean GFR of 126 mL/minute and an increase in UGE, whereas a 20% overexpression leads to a slightly increased mean GFR of 131 mL/minute and decreased UGE.

Simulated acute response to SGLT1/2 (co-)inhibition treatment

Potential effects of renal SGLT1i or combined SGLT1/2i treatment were assessed with the SHC and exemplary meal intake simulations. A healthy and an insulin-resistant individual were simulated for several days with three meals per day as described in **Data S1**.

The results of the simulations are presented in **Figure 4**. **Figure 4a,b** show simulated UGE and GFR during the SHC. The pharmacological effect of SGLT1i is qualitatively similar to that of SGLT2i but is quantitatively less prominent. A 50% inhibition of SGLT2 results in a higher amount of glucose excreted and stronger reduction of GFR than complete inhibition of SGLT1. The effect of SGLT1i is additive to that of SGLT2i.

In a healthy subject (**Figure 4c**), neither 50% inhibition of SGLT2 nor the total inhibition of SGLT1 has any prominent effect on the plasma glucose profile. Inhibition of SGLT2 by 100% is necessary to observe a modest reduction of GFR, and inhibition of SGLT1 has a strong GFR lowering effect when added to SGLT2i but not alone.

Figure 4d shows the results of the simulations with reduced insulin sensitivity. Without treatment, basal glucose concentration is 8 mmol/L, and the postprandial excursion reaches 14 mmol/L, values that would lead to the diagnosis of diabetes.[1] As predicted with SHC simulations, 50% SGLT2 or 100% SGLT1 inhibition have similar and only minor glucose lowering effects. Full inhibition of SGLT2 leads to almost complete normalization of fasting plasma glucose (6.5 mmol/L) and a reduction of GFR by 10 mL/minute. Addition of SGLT1i further reduces fasting plasma glucose to 6 mmol/L and lowers the GFR to ~115 mL/minute (-5 mL/minute compared to SGLT2i alone).

DISCUSSION

To our knowledge, we developed the first model that integrates regulation of glucose on the whole-body level with mechanism-based description of renal hyperfiltration and SGLT2i medication. Although other attempts to model hyperfiltration have been undertaken,[31] the *in silico* representation of the direct link between systemic glucose homeostasis and TGF remained unavailable.

Results of SHC simulation indicate some unexpected properties of GFR regulation and model behavior. The model predicts an increase of GFR proportional to plasma glucose concentrations up to ~11 mmol/L. This threshold associates with glucose concentrations at which net glucose excretion occurs. Although the maximal simulated GFR (132 mL/minute) may not be considered as hyperfiltration, the tubular cells come in contact with higher glucose load, and the glomeruli may be exposed to increased stress. Increase of plasma glucose above 11 mmol/L results in decreased GFR and is accompanied by decreased fractional water reabsorption. The simulated phenomenon seems to be caused by the countercurrent mechanism of water and sodium reabsorption in HL. When arterial glucose concentration becomes higher than 11 mmol/L, its reabsorption gets saturated, resulting in higher osmolality of the filtrate and retention of the water in the tubule. Water retention in PCT and PST leads to increased delivery of sodium to the ascending limb due to higher flow rate. The increased Na^+ amount results in increased reabsorption rate in the ascending limb, as the reabsorption is modeled as a first-order process. This causes an increase in interstitial sodium concentration and, therefore, increased water reabsorption in the descending limb of HL. Water reabsorption from the descending limb is not isoosmolar to sodium reabsorption in the ascending limb (20% of filtered sodium and 15% of filtered water).[9] The model calculates stronger proportional increase of water reabsorption in descending HL compared to sodium reabsorption increase in ascending HL, leading to relative increase in Na^+ concentration arriving at MD. Under SGLT2i treatment, increments of glucose concentration always lead to higher water retention in the tubule, and the simulated effect is comparable to that without treatment at plasma glucose above 11 mmol/L.

Therefore, results of SHC simulation indicate that osmolality of the filtrate has a major impact on GFR, as osmotic activity of glucose seems to counteract increased sodium reabsorption at some point. This observation stresses the importance of osmotic active substances in the filtrate and suggests that osmotic changes due to albuminuria must be considered in computational models of renal impairment.

As only mean GFR values were published for the SHC experiment, the authenticity of the simulated relationship between glucose concentrations and GFR cannot be fully validated. Should such data become available, further refinement or extension of the model can be performed. Ideally, the dataset would include precise plasma glucose concentrations during the clamp (including the eventual peaks produced by priming of each clamp step), UGE, and GFR (during baseline and at each clamp step) data on an individual level. This would not only allow estimating model parameters more precisely, but also assessing interindividual variability.

The complexity level of the presented model is sufficient to describe the process of interest—acute hyperglycemia driven increase of GFR—and the available data. However, one must be aware of the limitations resulting from the assumptions made. TGF, although it seems to be of major importance for the link between glycemia and GFR, is only a part of the complex GFR regulatory system. We did not include any neural or hormonal feedbacks on GFR. The model does not account for the systemwide regulation of sodium and water balance, and the predicted effects, therefore, may be only of acute nature, as they do not capture the possible regulation mechanisms activated by increased reabsorption during hyperglycemia. However, such sodium balance regulation probably have only minor implications on the TGF, as sodium balance is tightly regulated predominantly through controlled water reabsorption in the collecting duct (i.e., the post-MD sections of the nephron).[32] The long-term effects of SGLT2i on GFR may be underpredicted, as inhibition of SGLT2 causes downregulation of sodium-hydrogen exchanger 3, a transporter protein located in PT responsible for ~30% of Na^+ reabsorption.[33] Although clinical data do not indicate increased sodium excretion during dapagliflozin treatment,[27] such effect is observed with empagliflozin.[13] Indeed, there might be some differences between the mode of action of SGLT2i, and it might be necessary to substitute the unspecific Na^+ reabsorption by sodium-hydrogen exchanger 3 in the future to capture the renal hemodynamic effects of SGLT2i other than dapagliflozin. Further, the spatial structure of the kidney is highly complex and is simplified by the implemented compartmentalization. The model lumps the countercurrent mechanism to a single HL, as more complex modeling of the spatial heterogeneity would strongly increase complexity in the context of a whole-body PBPK model. We do not consider the regions located after the HL (i.e., the distal tubule and the collecting duct), and assume that the processes crucial for the regulation of TGF take place in the PT and HL. However, the distal tubule is involved in the regulation of water and Na^+ reabsorption and may be important for regulation of renal hemodynamics and GFR. Further limitations may arise from the fact that the model does not consider osmotic substances like urea.[9]

We compared the effects of acute increased Na^+ reabsorption due to hyperglycemia, overexpression of SGLT2, and kidney growth. We conclude that, according to our model, kidney growth is the crucial process in the development of hyperfiltration in early DM. Thus, our model supports the hypothesis of hyperfiltration development as it has been proposed by Vallon and Thomson[34] and is summarized in **Figure 5**. An initial increase of GFR, as predicted by the model during hyperglycemia, triggers a vicious cycle, resulting in higher delivery of glucose, insulin, and other growth factors, to the proximal tubule. Increased Na^+ reabsorption because of hyperglycemia seems not to be sufficient to solely account for the hyperfiltration. The growth of the proximal tubule can be considered as the crucial process, and could be caused by excess insulin as observed *in vitro*.[35] The manifestation of hyperfiltration implicates increased

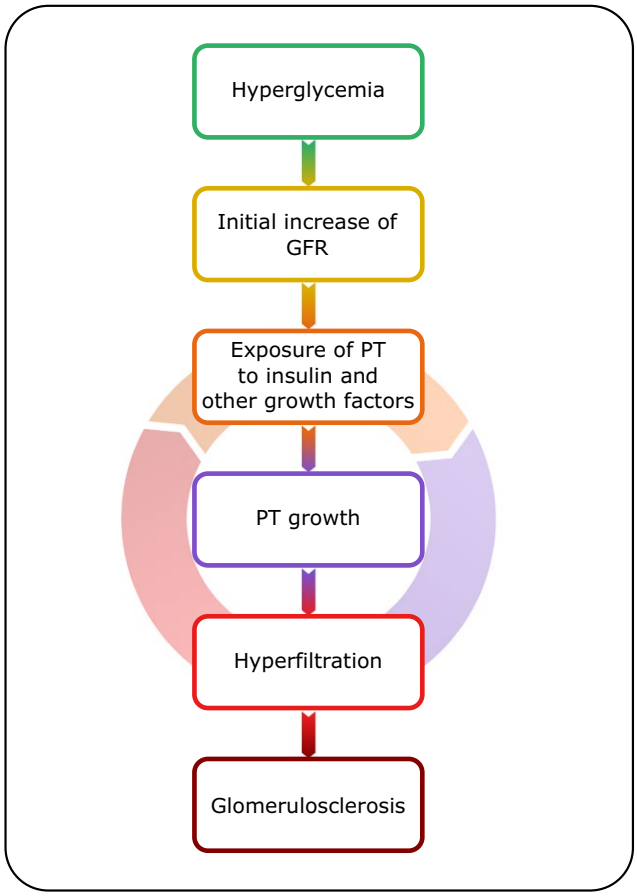

Figure 5 Hypothesis of the development of diabetes-induced hyperfiltration. Hyperglycemia triggers an initial increase of glomerular filtration rate (GFR), resulting in higher delivery of glucose, insulin, and other growth factors, to the proximal tubule (PT). A vicious cycle promotes the growth of PT and the manifestation of hyperfiltration. The resulting increased stress on glomeruli ultimately leads to glomerulosclerosis and loss of kidney function.

stress on glomeruli, ultimately leading to glomerulosclerosis and loss of kidney function. Of potentially high importance may be the predicted strong correlation between tubular size and GFR independently of plasma glucose concentration. We propose that model-based estimation techniques can be developed to earlier recognize hyperfiltration and CKD risks based on kidney size not only in diabetes. Overexpression of SGLT2, which could be induced by hyperglycemia,[14] did not show any strong effects on GFR and can, therefore, be considered as a reasonable biological response to high glucose levels. We consider the simulated 20% overexpression a reasonable assessment value, as much stronger overexpression would lead to markedly increased maximal glucose reabsorption capacity, whereas the observed elevation does not exceed 32% in patients with diabetes compared with healthy subjects.[15]

The developed dapagliflozin PBPK model accurately reproduces the observed effects on UGE in the simulations of typical daily food intake (**Figure S2**), and predicts the GFR lowering effect of SGLT2i comparable with that seen in patients with type 2 DM with moderate renal impairment.[10] The modeled connection between the systemwide glucose homeostasis and the local renal processes involving nonlinear dynamic feedback regulation has the potential to capture effects that are not straightforward and are not anticipated; one could think of the effects hyperglycemia may have on the PK of primarily GFR-cleared substances.

Simulations of the static inhibition of SGLT2 and SGLT1 allow estimation of the potential therapeutic benefit of renal SGLT1i, or the unspecific SGLTi. As expected, inhibition of renal SGLT1 alone cannot be considered as a potent medication for controlling blood glucose. Addition of SGLT1i to an SGLT2i improves glucose control to a small extent but has a prominent GFR reducing effect. Based on simulation results, we can propose that renal SGLT1i may be an effective treatment of hyperfiltration rather than hyperglycemia when administered with SGLT2i, which can be explained by the localization and properties of SGLT1. Located in the distal parts of the PT, SGLT1 only comes in contact with glucose when SGLT2 gets saturated. Therefore, inhibition of SGLT1 does not show any prominent effects without coadministration of SGLT2i. As SGLT1 transports two Na^+ per one glucose molecule, its inhibition has a stronger effect on sodium than on glucose reabsorption, reducing the GFR to a higher extent than blood glucose levels. Evidence exists that SGLT1 can transport Na^+ without the presence of glucose.[36] Should that be the case, SGLT1i could be potent GFR lowering agents even when administered alone.

The model confirms the proposed hypothesis of hyperglycemia-induced hyperfiltration. In the future, the model can be extended by a time-dependent glomerulosclerosis component to capture the progression of CKD in diabetes. In summary, we present a mathematical model of hyperfiltration coupled with glucose homeostasis, which is provided as **Data S2** and is available open source at www.open-systems-pharmacology.org, and allows researchers and physicians to study the pathology of the diabetic kidney and predict the outcomes of SGLTi medication and comedication with insulin and, potentially, other antidiabetic substances.

Supporting Information. Supplementary information accompanies this paper on the *CPT: Pharmacometrics & Systems Pharmacology* website (www.psp-journal.com).

Figure S1. Evaluation of the performance of the dapagliflozin PBPK model.
Figure S2. Simulations of the Diabetes Platform with extended kidney and dapagliflozin PBPK.
Table S1. Datasets used for model development
Table S2. Parameters of the dapagliflozin PBPK model
Table S3. Comparison of simulated vs. observed dapagliflozin concentration values
Data S1. Detailed model description.
Data S2. Model and simulation files.

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Author Contributions. P.B., S.S., T.E., and T.L. wrote the manuscript. SS., T.E., and T.L. designed the research. P.B. performed the research. P.B. and S.S. analyzed the data.

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