Prediction of the Effects of Renal Impairment on the Clearance for Organic Cation Drugs that

undergo Renal Secretion: A Simulation-Based Study

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Running Title: Effects of RI on renal secretion of OCT substrates

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Non-standard abbreviations – RI: renal impairment; RF: renal function; CL_R: renal clearance; MM: Michaelis-Menten; TDI: toxin drug interaction; DT: drug transporter; DME: drug metabolizing enzyme; GFR: glomerular filtration rate; PK: pharmacokinetics; PBPK: physiologically based pharmacokinetic model

Abstract:

Renal Impairment (RI) is a major health concern with a growing prevalence. RI leads to various physiological changes, in addition to a decrease in GFR, that impact the pharmacokinetics (PK) and specifically the renal clearance (CL_R) of compounds, including alterations of drug transporter (DT)/drug metabolizing enzyme expression and activity, as well as protein binding. The objectives of this study were to utilize the physiologically based pharmacokinetic (PBPK) modeling platform, Simcyp® to (1) assess the impact of alterations in DT expression, toxin-drug interactions (TDIs) and free fraction (f_u) on PK predictions for the OCT2/MATE1 substrate, metformin, in RI populations, and (2) utilize available *in vitro* data to improve predictions of Cl_R for two actively secreted substrates, metformin and ranitidine. The goal was to identify changes in parameters other than glomerular filtration rate, namely f_u, and DT expression/activity, that are consistent with *in vitro* and clinical data in RI, and predict the importance of these parameters on the PK of metformin and ranitidine in RI patients. Our results demonstrated that including alterations in DT expression and f_u, and including TDIs affecting DT activity, as indicated by *in vitro* data, improved the simulated predictions of CL_R and other PK parameters for both metformin and ranitidine in RI. Our simulations suggest that modifications of DT expression/activity and f_u are necessary for improved predictions of CL_R in RI for compounds that are actively secreted, and that improvement of PK predictions in RI populations for metformin and ranitidine can be obtained by incorporating *in vitro* data.

Introduction:

Renal impairment (RI) is a major health concern; the current prevalence of RI is 13.2% with a projected prevalence in 2030 of 16.7% in the United States alone (Hoerger et al., 2015). RI accompanies many other disease states including nephritis, glomerulonephritis, Type 2 diabetes and auto-immune diseases, such as lupus erythematosus (National Kidney Foundation, 2014). Since renal clearance (CL_R) is a major route of elimination for many drugs, kidney function is a relevant factor in the pharmacokinetics (PK) of these compounds. The FDA currently recommends clinical trials be conducted in RI populations for drugs where 30% or more of the drug is eliminated renally (FDA, 2010). Further characterization of the impact of RI on the PK of drugs is desirable because it may improve the accuracy of predictions for PK in RI populations.

RI is characterized by a decrease in the glomerular filtration rate (GFR), with decreasing estimates of GFR leading to more severe classes of RI (Inker et al., 2014). However, many other changes have been reported to occur in RI that are relevant to the clearance and distribution of drugs, including changes in drug transporter (DT) and drug metabolizing enzyme expression, as well as changes in protein binding (Keller et al., 1984; Vanholder et al., 1988; Dreisbach and Lertora, 2008; Nolin et al., 2008; Naud et al., 2011). In addition to these changes, some compounds are known to accumulate to toxic concentrations in RI, and some of these compounds have been shown to inhibit the activity of major DTs including members of the SLC and ABC families (Nigam et al., 2015; Katsube et al., 2017). One such compound, creatinine, has been shown to specifically inhibit transport via Organic Cation Transporter 2 (OCT2), a major secretory transporter located on the basolateral membrane in the kidney proximal tubule (Ciarimboli et al., 2012; Sauzay et al., 2016). The presence of elevated levels of uremic toxins introduces the possibility of toxin-drug interactions (TDIs), resulting in altered drug clearance.

The impact of RI on PK is of particular interest for drugs that are actively secreted in the kidney, such as metformin, an antidiabetic drug, and ranitidine, an H_2-receptor antagonist (Roberts, 1984; Gusler et al., 2001). Both metformin and ranitidine are actively transported from

the blood to the proximal tubule via OCT2, and subsequently out of the proximal tubule cell into the kidney lumen via multidrug and toxin extrusion protein 1 (MATE1) (Neuhoff, 2013; Hacker et al., 2015; Burt et al., 2016). OCT2 substrates are of particular interest as the FDA recommends testing drugs that are actively secreted in the kidney to determine if they are substrates for OCT2. (FDA, 2012). Metformin and ranitidine are well characterized in RI populations making them attractive model substrates for this study (Tucker et al., 1981; Zech et al., 1983; Roberts, 1984; Garg et al., 1986; Dixon et al., 1994; Sambol et al., 1995; Koch et al., 1997). It is currently difficult to predict potential changes in drug PK based on *in vitro* data and *in vivo* PK studies in normal volunteers. Using simulations based on metformin and ranitidine, which incorporate *in vitro* DT data as well as PK data in normal volunteers and RI patients, we can identify mechanisms that are important for the PK alteration in RI patients. This information will be important for further predictions for other OCT2/MATE1 substrates in assessing the potential changes in PK in RI.

Physiologically based pharmacokinetic (PBPK) modeling has been increasingly utilized for prediction of PK in specialized populations (Rowland et al., 2015). The PBPK modeling platform Simcyp® is useful for this approach, as it contains specialized populations, including those for RI. The advantage of PBPK platforms is they incorporate physiologically relevant information about specialized populations, including organ size and function and are able to incorporate additional information from *in vitro* studies.

The objectives of this work were to utilize the physiologically based pharmacokinetic modeling platform, Simcyp® to (1) assess the impact of alterations in glomerular filtration rate (GFR), DT expression and free fraction (f_u), as well as toxin-drug interactions (TDIs), on PK predictions for metformin in RI populations, and (2) utilize available *in vitro* data to improve predictions of renal clearance (CL_R) for two OCT2/MATE1 substrates, metformin and ranitidine. Simulated data will be compared to observed clinical data for these drugs in RI. These studies will identify significant changes in protein binding, GFR, DT expression/activity that are consistent

with in vitro and clinical data, and predictive of the PK alterations of metformin and ranitidine in RI

patients.

Materials and Methods:

Model for metformin in Simcyp®:

Simulations were performed in Simcyp® V15.1 simulator (Cerata), using the SV-metformin (3-(diaminomethylidene)-1,1-dimethylguanidine) substrate file. This model was developed and validated previously by Burt et al. 2016, the PBPK model scheme is presented in Supplementary Figure 1 and parameter values are available in the previous publication (Burt et al., 2016). This file was modified with regards to MATE1/2-K (SLC47A) transport, since the compound file did not contain J_{max} and K_M for MATE1/2-K transport, only the CL_{int} obtained from Ito et al. (Ito et al., 2012). Estimations for J_{max} and K_M were obtained from this same publication for MATE1, based on the Eadie-Hofstee plots for metformin transport in hMATE1 transfected HEK293 cells (Ito et al., 2012). The data was normalized per microgram protein, and then transformed to per million cells (the units compatible with Simcyp®) using the conversion 0.93 mg protein/10^6 HEK293 cells (Burt et al., 2016). An estimate for J_{max} was obtained from the y-intercept of digitized data (3245 pmol/min/10^6 cells) and the K_M was obtained from the negative slope of the data (306.2 pmol/μL). The estimates were utilized for MATE transport in the kidney, with the assumption that MATE1 transport would be dominant, and therefore drive the apical transport of metformin. This assumption was tested by running simulations using the estimated J_{max}/K_M for metformin and MATE1, compared with using the provided CL_{int} for metformin and MATE transport. The PK parameters agreed very well for both parametrizations; therefore, the estimated J_{max}/K_M for MATE1 were utilized for all simulations (Supplementary Table 1). This was done to allow for the manipulation of J_{max}/K_M in order to incorporate changes in DT expression and activity. The determined J_{max}/K_M was scaled to human kidney transport through the same relative activity factor (RAF) determined by Burt et al. via fitting (Burt et al., 2016). A representation of metformin transport in the kidney is provided in Figure 1.

Renal clearance is a result of several processes, including renal filtration (CL_{RF} or f_uGFR), renal secretion (CL_{RS}), and renal reabsorption (eq. 1). This study highlights the impact of

mechanistic modification of the renal secretion component of CL_R for compounds that are actively secreted, such as metformin and ranitidine.

$$CL_R = (f_u \cdot GFR + CL_{RS}) \cdot (1 - fraction\ reabsorbed) \qquad \text{eq. 1}$$

Dose Dependency of CL_R:

Simulations were performed utilizing both healthy and RI populations in Simcyp®. Sim-Healthy Volunteers, Sim_RenalGFR_30-60 (moderate RI) and Sim_RenalGFR_less_30 (severe RI) populations were used to represent GFR of 100, 50 and 10%, respectively. Two sets of simulations were performed, in exclusively male and female populations, to determine if there was a gender specific effect of RI as well. The doses utilized were 500, 1250, and 2000 mg.

Impact of DT Expression on CL_R:

In order to assess the impact of DT expression on the CL_R of metformin in the presence and absence of RI, simulations were performed with varying DT expression in healthy and RI populations. The same populations (Healthy Volunteers, Sim_RenalGFR_30-60 and Sim_RenalGFR_less_30) were used to represent healthy, moderate and severe RI with an approximate GFR of 100, 50 and 10%, respectively. DT expression changes were incorporated in the model indirectly by altering transporter function via J_{max} to reflect changes in protein expression (capacity). Both OCT2 and MATE1 were altered individually for each simulation by ±2- and ±5-fold. A single dose of 2000 mg metformin was administered in a fasted state. Simulations were performed separately in exclusively male and female populations, with each simulation including 10 trials of 10 individuals.

Impact of TDI on RI induced changes in CL_R:

TDIs were incorporated into the model in the form of inhibition through the manipulation of J_{max} and K_M. This was done with the parameter R, which is defined as the ratio of the concentration of the inhibitor (I), to its inhibition constant (K_i) (eq. 2). This parameter was used to include competitive, non-competitive and uncompetitive inhibition in the model via equations 3, 4 and 5, respectively.

$$R = \frac{[I]}{K_i} \quad \text{eq. 2}$$

$$v = \frac{J_{max}}{K_M(1+R)+S} \quad \text{eq. 3}$$

$$v = \frac{J_{max}/(1+R)}{K_M+S} \quad \text{eq. 4}$$

$$v = \frac{J_{max}/(1+R)}{K_M(1+R)+S} \quad \text{eq. 5}$$

R was varied from 0 to 100 to assess the impact of inhibition over a wide range of possible concentrations and potencies of inhibitors. Using this method, the degree of inhibition was constant throughout the simulation. This would be most relevant in the case of a TDI caused by a uremic toxin.

Simulations were performed with varying R values (0, 1, 10 and 100) for OCT2 and MATE1 separately. This resulted in four simulations being run for each transporter in each of the three populations representing varying renal function (Sim-Healthy Volunteers, Sim_RenalGFR_30-60 and Sim_RenalGFR_less_30). This set of 24 simulations was repeated for each type of inhibition. All simulations were performed for a single oral dose of 2000 mg metformin administered in a fasted state, and simulations included 10 trials with 10 subjects in each. Simulations were performed separately for male and female populations.

Impact of f_u on RI induced changes in CL_R with varying DT expression:

In order to assess the impact of potential changes in f_u in RI populations, simulations were performed with metformin with varying degrees of f_u, from 0 to 1. This was also assessed with varying degrees of DT (OCT2/MATE1) expression, in order to establish the potential relationship between these two factors. As with the TDI simulations, a single oral dose of 2000 mg metformin was administered in a fasted state, simulations included 10 trials with 10 subjects in each, and these were performed separately in male and female populations. Simulations were performed

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for varying f_u values (0.0, 0.1, 0.9 1.0) for each population (Sim-Healthy Volunteers, Sim_RenalGFR_30-60 and Sim_RenalGFR_less_30), resulting in a set of 12 simulations. This set of simulations was repeated with varying DT expression, by changing the J_{max} of OCT2/MATE1 together, ±2 and ±5 fold.

Impact of f_u on RI induced changes in CL_R in the presence and absence of TDI:

The impact of varying f_u was also investigated in the presence of TDI. As with the previously described TDI studies, competitive, non-competitive and uncompetitive inhibition was incorporated via the parameter R, as described through equations 2-5. Simulations were performed with Sim-Healthy Volunteers, Sim_RenalGFR_30-60 and Sim_RenalGFR_less_30 populations to represent GFR of 100, 50 and 10%, respectively. Inhibition was incorporated into the simulations through manipulation of J_{max}, K_M and J_{max}/K_M, for each transporter separately. For each population, f_u was varied (0, 0.1, 0.9 and 1.0) for each value of R (0, 1, 10, 100) tested, leading to a total of 48 simulations per transporter. A dose of 2000 mg metformin was administered in a fasted state to an exclusively male population; each simulation included 10 trials with 10 subjects in each. Simulations were not performed in a female population due to the observed similarity in trends and overall results between male and females.

Assessment of Clinical Relevance of RI changes:

In order to assess whether the potential additional changes in RI, beyond a decrease in GFR, were important to predictions of PK in RI populations, simulations were performed with the available RI populations in Simcyp® with additional changes supported by literature. Literature references of clinical data in healthy and RI populations were used for comparison for metformin and ranitidine ((E)-1-N'-[2-[[5-[(dimethylamino)methyl]furan-2-yl]methylsulfanyl]ethyl]-1-N-methyl-2-nitroethene-1,1-diamine) (Sambol et al., 1995; Koch et al., 1997). For the clinical ranitidine reference, PK parameters from groups 2 and 3 were averaged to represent the moderate RI population. The same was done for groups 4 and 5 to represent the severe RI population. The observed area under the curve (AUC) for ranitidine was calculated as dose/total

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clearance (CL), and the amount excreted into urine (A_e) was calculated as the ratio of CL_R to CL multiplied by dose. A previously developed compound file for ranitidine was utilized for simulations of ranitidine PK (Neuhoff, 2013). Simulations included 5 trials with 5 subjects each. For metformin, the reference did not state the proportion of female subjects in the study, and therefore a reasonable proportion of 0.34 was chosen for these simulations (Sambol et al., 1995). For ranitidine, the reported proportion of females was used (0.17) (Koch et al., 1997). For both metformin and ranitidine simulations, age ranges were manipulated to match the reference. Relevant PK parameters were obtained from simulations and compared to observed values.

Changes reported in literature relevant to metformin and ranitidine PK were then incorporated into the simulations and PK parameters were obtained for comparison. The relevant changes included a decrease in OCT2 expression to 20% of its original value for both metformin and ranitidine. This reduction in expression is based on protein expression data obtained in adenine-induced CRF rat kidneys (Komazawa et al., 2013). Changes in Organic Anion Transporter 3 (OAT3) were also included for ranitidine, as this transporter is also involved in the basolateral uptake of ranitidine in the kidney. OAT3 expression for ranitidine simulations was reduced to 10% of its original expression based on data from CRF rat kidneys (Komazawa et al., 2013). MATE1 expression was not reduced, as a transgenic mouse model of RI demonstrated no change in MATE1 mRNA in RI (Pedersen et al., 2016). Organic cation Transporter 1 (OCT1) mediates metformin uptake into the liver (Burt et al., 2016). OCT1 expression was shown to decrease to 10% of its original expression in adenine-induced CRF rat kidneys. While expression data was not available for OCT1 in the liver, simulations were performed to incorporate this reduction in OCT1 expression to investigate the impact of this transporter if hepatic and renal expression was affected in a similar manner by RI (Komazawa et al., 2013). Protein binding was not altered since both metformin and ranitidine are not extensively protein bound (Roberts, 1984; Burt et al., 2016).

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Finally, TDI was included for OCT2, OAT3 and for organic anion transporting polypeptide 1B3 (OATP1B3), a hepatic uptake transporter for ranitidine. This was based on available literature reports on potency of uremic toxins and the concentrations of these toxins in RI populations. Acrolein, a polyamine breakdown product and uremic toxin, was identified to inhibit OCT transport *in vitro* with a K_i of 93 ± 16 µM and demonstrating competitive or mixed-type inhibition (Schophuizen et al., 2013). The concentration of acrolein present in uremic populations has been reported to be 0.161 mM (total) or 1.35 µM (free). Based on these values, the R ($[I]/K_i$) would be 1.7 for total acrolein. An IC_{50} for creatinine inhibition of OCT2 has also been reported as 580 µM (Ciarimboli et al., 2012). The unbound concentration of creatinine in uremic patients is 1200 µM, and if IC_{50} is substituted for K_i, this would lead to an approximation of R of 2.0 (Hsueh et al., 2016). Therefore, competitive inhibition with an R of 2 was incorporated into the simulations as a potential TDI for OCT2.

For OAT3, several IC_{50} values have been identified for various uremic toxins, including creatinine (Hsueh et al., 2016). When compared with the concentrations of these toxins in uremic patients, the approximations of R over 1 were 1.3, 2.7 and 7.1 (Hsueh et al., 2016). For simplicity, competitive inhibition with an average R of 4 was incorporated into simulations as potential TDIs for OAT3. For hepatic uptake via OATP1B3, indoxyl sulfate has been demonstrated to inhibit methotrexate uptake in OATP1B3 expressing HEK293 cells (Sato et al., 2014). Sato et al. reported that indoxyl sulfate inhibited 40% of methotrexate transport at a concentration of 1000 µM (Sato et al., 2014). Since indoxyl sulfate has been reported to reach plasma concentrations as high as 940 µM, and if 1000 µM is approximated as the IC_{50} (this is the IC_{50} for the inhibition of methotrexate transport via OATP1B3 by indoxyl sulfate), this would lead to a possible approximation of R of 1 in RI populations (Vanholder et al., 2003; Sato et al., 2014). For this reason, competitive inhibition (R of 1) of ranitidine OATP1B3 hepatic uptake was investigated in this study. Other uremic toxins may be present in plasma concentrations that are greater than

their IC_{50} or K_i values, resulting in potential TDIs. This study was intentionally limited to potential changes for which there is supporting literature.

For metformin, the parameters assessed for improvement of prediction were time of maximal concentration (t_{max}), maximal concentration (C_{max}), AUC, CL_R, and A_e. For ranitidine, the parameters assessed for improvement of prediction were AUC, CL, CL_R and A_e. Improvement of prediction was based on the predicted/observed ratio for the mean parameter value. The absolute value of the difference between this ratio and 1 was determined (eq. 6) for each simulation, and is termed δ. This was computed for each parameter for each simulation, when δ was less than the value obtained with the base RI populations in Simcyp® (Sim_RenalGFR_30-60 and Sim_RenalGFR_less_30), there was considered to be an improvement in the prediction of that parameter. As δ approaches 0, the predicted value approaches the observed value.

$$\delta = \left| 1 - \frac{predicted}{observed} \right| \quad \text{eq. 6}$$

In order to assess the improvement in the overall prediction for a specific simulation, the δ for each parameter was summed and compared to the sum for the base RI populations. When the sum of δ for all parameters ($\Sigma\delta_{all}$) was less than that of $\Sigma\delta_{all}$ for the base RI population, this was considered to be an improvement in the overall prediction.   This was also assessed separately for the renal clearance parameters, Cl_R and A_e, as the sum of δ for CL_R and A_e alone, and is termed $\Sigma\delta_R$. When the $\Sigma\delta_R$ for a particular simulation was less than $\Sigma\delta_R$ for the base RI population, this was considered to be an improvement in the prediction of renal clearance, specifically. The results as discussed in terms of which conditions led to an improvement for each parameter, which conditions led to the three lowest $\Sigma\delta_{all}$, and which led to the lowest $\Sigma\delta_R$.

Results:
Dose Dependency of CL_R:

The CL_R of metformin did not exhibit dose-dependency. CL_R decreased as RI increased (i.e. GFR was decreased). There was an approximate 1.8-fold reduction in CL_R when the severe

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RI population was used as opposed to the healthy population, regardless of dose (Fig. 2, Supplementary Table 2). While values for CL_R were slightly lower for females than males, the trend of the impact of RI, as well as the magnitude of the impact of changes, remained consistent for both sexes. Figures and tables regarding the results in females are provided for completeness in the supplementary information for all evaluations (Supplementary Fig. 5-8, Supplementary Tables 30-38).

Impact of DT Expression on CL_R:

CL$_R$ increased as DT expression increased and decreased as DT expression decreased (Fig. 3, Supplementary Table 3). In a healthy population, when DT expression was increased 5-fold, CL_R was increased 1.6-fold (Fig. 3, Supplementary Table 3). The impact of altering DT expression increased as renal function decreased. For a severe RI population, when DT expression was increased 5-fold, Cl$_R$ was increased 2.2-fold (Fig. 3, Supplementary Table 3). When DT expression was decreased 5-fold in a healthy and severe RI population respectively, CL_R decreased 2.1- and 2.9-fold (Fig. 3, Supplementary Table 3).

Impact of TDI on CL_R:

The impact of all types of inhibition on the CL_R of metformin was greater for OCT2 than for MATE1, the results for non-competitive inhibition are shown in Figure 4. Competitive and non-competitive inhibition yielded almost identical results for OCT2 inhibition, with maximum inhibition tested (R of 100) resulting in 4-, 6- and 9-fold reductions in CL_R for healthy, moderate and severe RI populations, respectively (Fig. 4A and Supplementary Fig. 2A, Supplementary Table 4 and 5). OCT2 uncompetitive inhibition had slightly greater effects, compared to competitive and non-competitive inhibition, with 4, 7 and 11 fold reductions in CL_R for healthy, moderate and severe RI populations, respectively (Supplementary Fig. 2B, Supplementary Table 6).

Competitive inhibition of MATE1 resulted in no change in CL_R, or any other PK parameter (Supplementary Fig. 2C, Supplementary Table 4). Non-competitive inhibition had a slight effect, leading to 1.2-, 1.6- and 2-fold reductions in CL_R for healthy, moderate and severe RI populations,

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respectively (Fig. 4B, Supplementary Table 5). As with OCT2, the effects of uncompetitive inhibition were slightly greater than those of non-competitive inhibition, with reductions in CL_R of 1.4-, 1.8- and 2.2-fold for healthy, moderate and severe RI populations (Supplementary Fig. 2D, Supplementary Table 6).

Impact of f_u on RI induced changes in CL_R with varying DT expression:

When f_u was increased from 0.1 to 1 in a healthy population, CL_R of metformin increased 5.6-fold (Fig. 5A, Supplementary Table 7). The effect of increasing f_u was slightly more pronounced as renal function decreased, with the moderate and severe RI populations resulting in 5.8- and 5.9-fold increasing in CL_R for the same change in f_u (Fig.5A , Supplementary Table 7). When DT expression was increased, the effect of f_u on CL_R was minimized. When DT expression was increased 5-fold and f_u was increased from 0.1 to 1.0, CL_R increased 2.7-, 3.0- and 3.3-fold for healthy, moderate and severe RI populations, respectively (Fig. 5C, Supplementary Table 8). Again, as renal function decreased, f_u had a greater effect on CL_R.

When DT expression was decreased 5-fold, the effect of f_u on CL_R was magnified; for all degrees of RI, CL_R was increased an average of 8-fold when f_u was increased from 0.1 to 1.0 (Fig. 5E, Supplementary Table 9). When DT expression was decreased, unlike normal and increased expression, the effect of f_u decreased slightly as renal function decreased. CL_R increased 8.5-fold for the healthy population when f_u increased from 0.1 to 1.0, but increases were slightly less (8.2- and 7.7-fold) for moderate and severe RI populations, respectively (Fig. 5E, Supplementary Table 9). Similar effects were observed when DT expression was altered ±2-fold (Fig. 5B and 5D, Supplementary Tables 10 and 11).

Impact of f_u on RI induced changes in CL_R in the presence and absence of TDI:

For both OCT2 and MATE1, the greatest reduction in CL_R was observed with uncompetitive inhibition and when f_u was minimal (Figs. 6 and 7, Supplementary Tables 12-17). As f_u was increased, with the same degree of inhibition (R), the impact of inhibition of CL_R was minimized. When f_u was minimal (0.1), maximal uncompetitive inhibition of OCT2 (R of 100) led

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to a reduction in CL_R of metformin of 7-, 10-, and 15-fold for healthy, moderate and severe RI populations (Fig. 6, Supplementary Tables 12-14). For the same degree of inhibition and renal impairment when f_u was increased to its maximal value (1.0), CL_R was reduced 4-, 7-, and 11-fold. For uncompetitive inhibition of MATE1, the reduction in CL_R for healthy, moderate and severe RI populations was 5.5-, 6.9- and 7.7-fold when f_u was minimal (0.1), and 1.4-, 1.7- and 2.1-fold when f_u was maximal (1.0) (Fig. 7, Supplementary Tables 15-17).

Competitive and non-competitive inhibition yielded very similar results, and the trend with regards to f_u was the same as uncompetitive inhibition. When f_u was minimal (0.1), maximal competitive and non-competitive inhibition of OCT2 (R of 100) led to a 7-, 9- and 13-fold reduction in CL_R for healthy, moderate and severe RI populations, respectively (Supplementary Fig. 3, Supplementary Tables 18-23). For the same type and degree of inhibition, and degree of renal impairment, CL_R was reduced 4-, 6-, and 9-fold when f_u was maximal (1.0).

For MATE1, competitive inhibition did not impact CL_R, regardless of changes in f_u (Supplementary Fig. 4A-C, Supplementary Tables 24-26). For non-competitive and uncompetitive inhibition of MATE1, the same trend was observed where an increase in f_u minimized the effect of inhibition on CL_R (Fig. 7 and Supplementary Fig. 4D-F). Again, this effect was much more pronounced for uncompetitive inhibition than non-competitive inhibition. For non-competitive inhibition of MATE1, the reduction in CL_R for healthy, moderate and severe RI populations was 1.4-, 1.7- and 2.2-fold when f_u was minimal (0.1), and 1.2-, 1.5- and 1.9-fold when f_u was maximal (1.0) (Supplementary Fig. 4D-F, Supplementary Tables 27-29).

Assessment of Clinical Relevance of RI changes:

For metformin, δ was lower for the majority of simulations that included additional changes in RI with respect to OCT2 and OCT1 expression and function compared to the simulations for the base RI populations (Table 1 and Supplementary Table 39). The parameter for which there was the least improvement was A_e; this was true for both moderate and severe RI, but was more pronounced with severe RI (Table 1 and Supplementary Table 39). This was due to the base

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severe RI population producing an accurate prediction of A_e with a δ of only 0.036 (Table 1). For both RI populations, inclusion of a modification in OCT2 transport resulted in the best overall prediction and prediction of renal clearance parameters (lowest $\Sigma\delta_{all}$ and $\Sigma\delta_R$, respectively). For moderate RI, the inclusion of a reduction in OCT2 expression to 20% of its original value resulted in the best predictions (4- and 10-fold reduction for $\Sigma\delta_{all}$ and $\Sigma\delta_R$, respectively), while inclusion of inhibition of OCT2 (R of 2), not an expression change, resulted in the best prediction for severe RI (3- and 6-fold reduction for $\Sigma\delta_{all}$ and $\Sigma\delta_R$, respectively) (Table 1). The improvement in prediction of observed clinical data is presented in Figure 8, where the prediction by the base Simcyp RI population is plotted for both moderate and severe RI (Fig. 8A and 8C). The most improved simulation, determined by the most improvement in parameter predictions given in Table 1, are plotted for moderate and severe RI (Fig. 8B and 8D). While the observed data is not completely predicted, most notably there is an under-prediction of later time points for all simulations, the inclusion of expression changes and inhibition of OCT2 does lead to an improvement of prediction for both RI populations.

For ranitidine, inclusion of expression and activity changes for OCT2, OCT1 and OATP1B3 did result in some degree of improvement for both populations (Table 2 and Supplementary Table 40). More improvement was observed for the severe RI population; δ was lower for the majority of simulations for severe RI populations when compared to the δ for the base RI population provided in Simcyp®. Fewer improvements were observed for the moderate RI populations, as the base moderate RI population provided reasonably accurate estimates for the PK parameters. The greatest improvements for the moderate RI population were observed in the CL_R and A_e parameters for ranitidine, (Table 2 and Supplementary Table 40). As with metformin, modification of the expression and activity of OCT2 led to the greatest improvements in CL_R and A_e for ranitidine. The greatest improvement was seen with the inclusion of inhibition of OCT2 (R of 2), with a $\Sigma\delta_R$ of 0.142, followed by a decrease in the expression of OCT2 to 20% of its original value with a $\Sigma\delta_R$ of 0.187 (Table 2). These two conditions led to a decrease in $\Sigma\delta_R$

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of over 4- and 3-fold when compared to the $\Sigma\delta_R$ for the base moderate RI population, respectively (Table 2). The greatest improvement in overall prediction of the PK parameters was observed with modification of OAT3, not OCT2. When OAT3 expression was reduced to 10% of its original value and inhibition was included (R of 4), the lowest value for $\Sigma\delta_{all}$ was achieved. This set of conditions also led to a similar improvement in $\Sigma\delta_R$ as was achieved with reduction of OCT2 expression, with a $\Sigma\delta_R$ of 0.188 (Table 2).

For the severe RI population, nearly every simulation which included expression and activity changes for OCT2, OCT1 and OATP1B3 resulted in a reduction in δ for every parameter when compared to the base severe RI population δ (Table 2 and Supplementary Table 40). The conditions that improved the $\Sigma\delta_{all}$ and $\Sigma\delta_R$ were mainly identical between the two doses tested (50 and 25 mg). The lowest values for $\Sigma\delta_{all}$ and $\Sigma\delta_R$ were achieved when the expression and activity of both OCT2 and OAT3 were reduced. Expression was reduced to 20 and 10% respectively, for OCT2 and OAT3, and inhibition was included for both transporters with an R of 2 and 4, respectively. When these changes were included, $\Sigma\delta_{all}$ and $\Sigma\delta_R$ were both reduced by approximately 30- and 5-fold for the 50 and 25 mg dose, respectively when compared to $\Sigma\delta_{all}$ and $\Sigma\delta_R$ for the predictions obtained from the base severe RI population in Simcyp® (Table 2). The next most accurate simulation was obtained with the same conditions, with the removal of the inhibition of OAT3. For this simulation, $\Sigma\delta_{all}$ and $\Sigma\delta_R$ were both reduced by approximately 21- and 4- fold for the 50 and 25 mg dose, respectively when compared to $\Sigma\delta_{all}$ and $\Sigma\delta_R$ for the predictions obtained from the base severe RI population in Simcyp® (Table 2). The most improved predictions are depicted in Figure 9 for ranitidine. The base RI populations for moderate and severe RI provide a reasonable prediction of the ranitidine plasma profile; however, inclusion of additional conditions in RI did lead to an improvement in prediction for both RI populations. The predictions given by the Simcyp® moderate and severe RI populations are given in Figure 9A and 9C, respectively. The most improved simulation, as indicated in Table 2, is plotted for moderate RI and includes a reduction in OAT3 expression to 10% of normal, and competitive inhibition of OAT3

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with an R of 4 (Fig. 9B). For severe RI, the most improved simulation included the same

modification of OAT3 as with moderate RI (10% and R4 OAT3), along with a reduction in OCT2

expression (20% of normal) and competitive inhibition of OCT2 (R of 2) (Fig. 9D).

Discussion:

RI is a major and growing health concern worldwide which can occur alone, or can accompany a number of disease states (National Kidney Foundation, 2014; Hoerger et al., 2015). While a decrease in GFR characterizes this disease state, numerous other changes can occur within RI that have the potential to alter the PK of compounds. These changes are of particular interest for drugs that are actively secreted in the kidney, as alterations in expression of major drug transporters in the kidney, as well as inhibition of these transporters with accumulated uremic toxins in RI have been reported (Vanholder et al., 2003; Dreisbach and Lertora, 2008; Nolin et al., 2008; Naud et al., 2011; Ciarimboli et al., 2012; Komazawa et al., 2013; Schophuizen et al., 2013; Sato et al., 2014; Hsueh et al., 2016; Sauzay et al., 2016). Beyond changes in transporter expression and activity, changes in drug metabolizing enzyme expression and activity, as well as modifications in protein binding can occur in RI (Keller et al., 1984; Vanholder et al., 1988; Dreisbach and Lertora, 2008; Nolin et al., 2008; Naud et al., 2011). In order to assess the potential impact of these changes, this study utilized Simcyp® and two well characterized model substrates which undergo active secretion in the kidney involving OCT2, and MATE1, metformin and ranitidine.

The impact of dose, DT expression, TDIs, and the impact of altering f_u along with DT expression and TDIs were systematically investigated utilizing metformin, in order to establish quantitative relationships for the impact of these factors on CL_R and overall PK for metformin. Neither the dose of metformin nor the sex of individuals in the trials altered the impact of RI on the predictions of metformin PK. This is a significant finding as any trends that are determined to be vital to the proper prediction of metformin PK in RI populations will not be affected by these factors.

As expected, renal DT expression had an impact on the CL_R of metformin. As DT expression was decreased, there was an accompanying decrease in CL_R of metformin, as the active secretion component of CL_R was minimized. The impact was greater when renal function

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was reduced. This suggests that any observed changes in DT expression in RI populations will be an increasingly important factor in prediction of PK as renal function decreases. The same trend was seen with inhibition of OCT2 and MATE1, as renal function was decreased, the impact of the same degree of inhibition resulted in greater decreases in CL_R. TDIs are likely to be more severe as renal function declines. The accumulation of uremic toxins occurs with declining renal function; therefore, the CL_R may be more drastically reduced than expected, based on a reduction in GFR in RI populations, if interactions with uremic toxins occurs with actively secreted compounds. It is also of note that the type of inhibition (competitive, noncompetitive, uncompetitive) did not lead to very different estimates of metformin PK, suggesting the type of inhibition is not a major factor in the determining the impact of TDIs on PK.

Overall, our simulations indicated that inhibition of OCT2 played a much larger role in metformin PK than inhibition of MATE1. This may suggest that OCT2 transport is the rate-limiting step in the active secretion of metformin. However, cimetidine, a more potent inhibitor of MATE1 than OCT2, has been shown to clinically alter the CL_R of metformin (Somogyi et al., 1987; Wang et al., 2008). This discrepancy may be explained by investigating the interplay between metformin efflux via MATE1 and influx via OCT2. OCT2 is an electrogenic transporter, and reduction of metformin efflux has been shown to increase the concentration of metformin and thereby reduce the driving force for metformin uptake via OCT2 in the proximal tubule (Burt et al., 2016). Incorporation of the electrogenic nature of OCT2 results in an increase in metformin plasma concentrations when MATE1/2-K inhibition is included in simulations (Burt et al., 2016). This is consistent with our findings using the conventional (non-electrogenic) version of the metformin model in Simcyp® that OCT2 inhibition is an important predictor of CL_R of metformin and MATE1 inhibition, on its own, is not. MATE1 inhibition would likely prove to be more significant when its effect on OCT2 transport is incorporated.

RI may cause a decrease in protein binding due to uremia, hypoalbuminemia, or drug interactions (Keller et al., 1984). In this study we varied f_u from 0.1 to 1.0 in order to investigate

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the maximal effects of potential changes in f_u, despite the fact that metformin is negligibly protein bound even in healthy populations (Burt et al., 2016). As f_u was increased, there was a large increase in Cl_R which was magnified as renal function decreased. This indicates that changes in f_u may have a greater effect as RI progresses. When DT expression was increased, the effect of f_u on CL_R was minimized, while when DT expression was decreased, the effect of f_u was magnified. This indicates that DT expression can modulate the effect of f_u on CL_R. In a case, such as metformin, where OCT2 is a major DT that contributes to its CL_R, if OCT2 expression decreases, any changes in f_u may be magnified and play a more significant role than if normal expression was expected.

For changes in f_u with concurrent TDI, it was observed that an increase in f_u minimized the impact of inhibition on reducing CL_R. When maximal inhibition was included in the simulation, the reduction in CL_R was reduced when f_u was increased. This suggests that if a TDI is expected to occur in RI, the expected reduction in CL_R may be over-predicted if the f_u of the drug of interest is also increased in RI. Incorporation of changes in protein binding, when they are expected to be significant, may lead to more accurate predictions of overall PK and CL_R.

In order to improve the predictions of PK in RI populations, *in vitro* data was incorporated into the model for both metformin and ranitidine. For these simulations, a reduction in the activity and expression of OCT2 was included for both metformin and ranitidine (Ciarimboli et al., 2012; Komazawa et al., 2013; Schophuizen et al., 2013). Alterations in MATE1 transport were not included in simulations for comparison to clinical data as no change in mRNA was observed in a transgenic mouse model of RI (Pedersen et al., 2016). Alterations in OAT3 were also included in simulations for ranitidine, as this transporter is involved in the renal uptake of the compound (Komazawa et al., 2013; Hsueh et al., 2016). Inhibition of hepatic transport for metformin (OCT1) and ranitidine (OATP1B3) was also evaluated (Vanholder et al., 2003; Ciarimboli et al., 2012; Komazawa et al., 2013; Schophuizen et al., 2013; Sato et al., 2014).

For metformin, including modifications of OCT2 expression and activity led to the most accurate predictions of overall PK and of renal clearance parameters (CL_R and A_e). For moderate RI, reducing OCT2 expression led to the most accurate prediction, while inhibiting OCT2 transport led to the most accurate predictions for severe RI.

For ranitidine the base moderate RI population in Simcyp® provided a reasonably accurate prediction of ranitidine overall PK; despite this, improvements were still observed with the inclusion of some of these additional changes, especially for the renal clearance parameters CL_R and A_e. As with metformin, alterations in OCT2 expression and activity led to the two best predictions of CL_R and A_e for moderate RI. However, alterations in OAT3 expression and activity led to the best predictions of overall PK. This suggests that the impact of alteration of OAT3, not OCT2, in RI may be more significant for ranitidine at least when RI is still moderate. For the severe RI populations, inclusion of additional changes in DT expression and activity resulted in an almost universal improvement in prediction over the base severe RI population in Simcyp®. The conditions for simulation which provided the most accurate predictions of overall PK and for CL_R and A_e, were identical for both doses and included a decrease in both OCT2 and OAT3 expression, as well as inhibition of both of these transporters. These results highlight the importance of including the impact of RI on OCT2 and OAT3 in order to produce accurate predictions of ranitidine PK. Due to the changes in transporter expression induced in RI, it is possible that some compensatory mechanisms occur. This possibility was not explored in the current study.

This study highlights the importance of understanding the impact of RI on CL_R on a mechanistic level with regards to DT expression, TDIs and f_u in RI populations (Figure 10). The most accessible way to obtain mechanistic information about these factors is through *in vitro* studies, and we have demonstrated the usefulness of this data in improving the predictions of CL_R for two OCT2/MATE1 substrates, metformin and ranitidine, in clinical RI populations. This

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information will enable more accurate prediction of other OCT2/MATE1 substrates and a similar

approach may allow for more accurate predictions for substrates of other transporters.

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Authorship Contributions:

Participated in research design: Follman and Morris.

Conducted experiments: Follman.

Performed data analysis: Follman and Morris

Wrote or contributed to the writing of the manuscript: Follman and Morris.

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Legends for Figures:

Figure 1. Schematic Representation of Metformin Transport in the Kidney. OCT2 and MATE1 participate in the active secretion of metformin. Metformin is taken up from the blood via OCT2 and transported into the proximal tubule cell. Metformin is effluxed out of the proximal tubule cell via MATE1 and MATE2-K.

Figure 2. Impact of Renal Impairment (RI) on the Dose Dependency of Metformin. Simulations were performed in Simcyp® with oral metformin at 500, 1250 and 2000 mg. Simulations were performed in a male population with healthy and RI populations. Sim-Healthy Volunteers, Sim_RenalGFR_30-60 and Sim_RenalGFR_less_30 populations were used to represent GFR of 100, 50 and 10%, respectively. Each simulation included 10 trials with 10 subjects in each.

Figure 3. Impact of Renal Impairment (RI) and Drug Transporter Expression on the Renal Clearance of Metformin. Simulations were performed in Simcyp® with Sim-Healthy Volunteers, Sim_RenalGFR_30-60 and Sim_RenalGFR_less_30 populations to represent GFR of 100, 50 and 10%, respectively. A dose of 2000 mg metformin was administered in a fasted state to an exclusively male population. Expression of OCT2 and MATE1 was changed by altering J_{max} ±2- or ±5-fold. Each simulation included 10 trials with 10 subjects in each.

Figure 4. Effect of Renal Impairment (RI) and Inhibition on the Renal Clearance of Metformin in Males. Simulations were performed in Simcyp® with Sim-Healthy Volunteers, Sim_RenalGFR_30-60 and Sim_RenalGFR_less_30 populations to represent GFR of 100, 50 and 10%, respectively. A dose of 2000 mg metformin was administered in a fasted state to an exclusively male population. Inhibition was incorporated through the parameter, R ($[I]/K_i$), which modified K_M or J_{max} for competitive and non-competitive inhibition, respectively. R was varied from 0 to 100 to investigate varying levels of inhibition. Inhibition was applied to each transporter (OCT2/MATE1) separately. A non-competitive inhibition of OCT2; B non-competitive inhibition of MATE1. Each simulation included 10 trials with 10 subjects in each.

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Figure 5. Effect of Renal Impairment (RI) and Fraction Unbound on the Renal Clearance of Metformin with Varying Expression of OCT2 and MATE1 in Males. Simulations were performed in Simcyp with Sim-Healthy Volunteers, Sim_RenalGFR_30-60 and Sim_RenalGFR_less_30 populations to represent GFR of 100, 50 and 10%, respectively. A dose of 2000 mg metformin was administered in a fasted state to an exclusively male population. Fraction unbound was varied from 0.1 to 1.0. Expression of OCT2 and MATE1 was altered by increasing or decreasing J_{max} 2 or 5 fold. A, original expression (J_{max}) of OCT2/MATE1; B/D, ± 2-fold expression (J_{max}) of OCT2/MATE1; C/E, ± 5-fold expression (J_{max}) of OCT2/MATE1. Each simulation included 10 trials with 10 subjects in each.

Figure 6. Effect of Renal Impairment (RI) and Fraction Unbound on the Renal Clearance of Metformin with Accompanying Inhibition of OCT2. Simulations were performed in Simcyp with Sim-Healthy Volunteers (A), Sim_RenalGFR_30-60 (B) and Sim_RenalGFR_less_30 (C) populations to represent GFR of 100, 50 and 10%, respectively. A dose of 2000 mg metformin was administered in a fasted state to an exclusively male population. Fraction unbound was varied from 0.1 to 1.0. R ($[I]/K_i$), which modified K_M and J_{max} for uncompetitive inhibition. R was varied from 0 to 100 to investigate varying levels of inhibition. Each simulation included 10 trials with 10 subjects in each.

Figure 7. Effect of Renal Impairment (RI) and Fraction Unbound on the Renal Clearance of Metformin with Accompanying Inhibition of MATE1. Simulations were performed in Simcyp with Sim-Healthy Volunteers (A), Sim_RenalGFR_30-60 (B) and Sim_RenalGFR_less_30 (C) populations to represent GFR of 100, 50 and 10%, respectively. A dose of 2000 mg metformin was administered in a fasted state to an exclusively male population. Fraction unbound was varied from 0.1 to 1.0. R ($[I]/K_i$), which modified K_M and J_{max} for uncompetitive inhibition. R was varied from 0 to 100 to investigate varying levels of inhibition. Each simulation included 10 trials with 10 subjects in each.

Figure 8. Metformin Plasma Profiles for Observed and Simulated Renally Impaired Populations. Observed plasma concentrations for metformin were plotted (black dots) for both moderate (A, B) and severe (C,D) renally impaired populations following an 850 mg oral dose of metformin obtained from Sambol et al. 1995 (Sambol et al., 1995). The mean, 5th and 95th percentile data from simulations with Simcyp were then overlaid with the observed data for comparison. Simulations were performed with demographics and study conditions to match Sambol et al. 1995. The pictured simulations include the base Simcyp RI population, and the most improved simulation as determined by improvement in the prediction/observed values for parameters given in Table 1. A. Sim_RenalGFR_30-60 B. Sim_RenalGFR_30-60 with 20% OCT2 expression (compared to normal expression) C. Sim_RenalGFR_less_30 D. Sim_RenalGFR_less_30 with OCT2 inhibition of R2 (competitive). Each simulation included 10 trials with 10 subjects in each.

Figure 9. Ranitidine Plasma Profiles for Observed and Simulated Renally Impaired Populations. Observed plasma concentrations for ranitidine were plotted (black dots) for both moderate (A, B) and severe (C,D) renally impaired populations following a 50 mg intravenous dose of ranitidine obtained from Koch et al 1997 (Koch et al., 1997). The mean, 5th and 95th percentile data from simulations with Simcyp were then overlaid with the observed data for comparison. Simulations were performed with demographics and study conditions to match Koch et al. 1997. The pictured simulations include the base Simcyp RI population, and the most improved simulation as determined by improvement in the prediction/observed values for parameters given in Table 2. A. Sim_RenalGFR_30-60 B. Sim_RenalGFR_30-60 with 10% OAT3 expression (compared to normal expression) and OAT3 inhibition of R2 (competitive) C. Sim_RenalGFR_less_30 D. Sim_RenalGFR_less_30 with 10% OAT3 expression (compared to normal expression), OAT3 inhibition of R2 (competitive), 20% OCT2 expression (compared to normal expression), and OCT2 inhibition of R2 (competitive). Each simulation included 10 trials with 10 subjects in each.

Figure 10. Summary of the Potential Physiological Impact of Renal Impairment (RI) on Metformin and Ranitidine. Simulations show that for the probe substrate metformin, a decrease

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in protein binding leads to an increase in renal clearance (CL_R), while a decrease in GFR, drug

transporter (DT) expression, and toxin drug interactions (TDIs) all lead to a decrease in CL_R.

Inclusion of a decrease in DT expression and TDIs also lead to an improvement of clinical PK in

RI populations for metformin and ranitidine.

Table 1. Comparison of predicted and observed values for PK parameters for Metformin. Simulation conditions are listed by rank order according to $\Sigma\delta_{all}$. δ is the absolute difference between one and the ratio of the predicted value of the parameter over the observed value, as defined by equation 6 in the methods section. Supplementary Table 39 provides actual parameter estimates as well as δ values.

Table 1. Comparison of predicted and observed values for PK parameters for Metformin	δ					$\Sigma\delta_{all}$	$\Sigma\delta_R$
	t_{max}	C_{max}	AUC	CL_R	A_e		
Healthy							
Sim Healthy Volunteers	0.191	0.007	0.172	0.162	0.062	0.595	0.224
Moderate RI							
Sim GFR 30-60	0.328	0.536	0.674	2.15	0.249	3.94	2.40
OCT2 20%	0.040	0.347	0.374	0.192	0.056	1.01	0.248
OCT2 20% OCT2 R2	0.152	0.262	0.185	0.303	0.234	1.14	0.537
OCT2 20% OCT1 10%	0.248	0.150	0.038	0.191	0.524	1.15	0.715
OCT2 20% OCT1 10% OCT2 R2	0.536	0.000	0.470	0.303	0.361	1.67	0.664
OCT2 R2	0.136	0.403	0.477	0.631	0.048	1.69	0.679
OCT1 10% OCT2 R2	0.088	0.243	0.197	0.631	0.565	1.72	1.20
OCT1 10%	0.232	0.439	0.559	2.15	0.654	4.04	2.81
Severe RI							
Sim GFR less than 30	0.282	0.463	0.563	1.10	0.036	2.45	1.14
OCT2 R2	0.012	0.310	0.278	0.018	0.186	0.806	0.204
OCT1 10% OCT2 R2	0.197	0.102	0.197	0.018	0.304	0.818	0.322

OCT2 20%	0.077	0.247	0.131	0.317	0.297	1.07	0.614
OCT2 20% OCT1 10%	0.347	0.008	0.608	0.318	0.248	1.53	0.566
OCT2 20% OCT2 R2	0.167	0.160	0.133	0.644	0.487	1.59	1.13
OCT1 10%	0.162	0.341	0.377	1.10	0.428	2.41	1.53
OCT2 20% OCT1 10% OCT2 R2	0.676	0.181	1.235	0.645	0.000	2.74	0.645

Table 2. Comparison of predicted and observed values for PK parameters for Ranitidine

Simulation conditions are listed by rank order according to Σδ$_{all}$. δ is the absolute difference

between one and the ratio of the predicted value of the parameter over the observed value, as

defined by equation 6 in the methods section. This table has been abbreviated to contain only

the top five simulations for each dose/RI combination. Supplementary Table 40 provides values

for each simulation including actual parameter estimates as well as δ values.

Table 2. Comparison of predicted and observed values for PK parameters for Ranitidine	Dose mg	δ				Σδ$_{all}$	Σδ$_R$
		AUC	CL	CL$_R$	A$_e$		
Healthy							
Sim Healthy Volunteers	50	0.34	0.235	0.400	0.160	1.14	0.560
Sim Healthy Volunteers	25	0.53	0.331	0.487	0.179	1.53	0.666
Moderate RI							
Sim GFR 30-60		0.083	0.090	0.360	0.256	0.789	0.616
OAT3 10% OAT3 R4		0.083	0.075	0.060	0.128	0.346	0.188
OAT3 10%	50	0.067	0.060	0.090	0.140	0.357	0.230
OAT3 R4		0.044	0.045	0.120	0.156	0.365	0.276
OCT2 R2		0.139	0.120	0.066	0.076	0.401	0.142
OCT2 20%		0.206	0.170	0.167	0.020	0.563	0.187
Severe RI							
Sim GFR less than 30		0.423	0.692	3.95	2.27	7.33	6.22
OCT2 20% OAT3 10% OCT2 R2 OAT3 R4		0.019	0.037	0.069	0.129	0.254	0.198
OCT2 20% OAT3 10% OCT2 R2	50	0.006	0.019	0.059	0.254	0.338	0.313
OCT2 20% OCT2 R2 OAT3 R4		0.035	0.019	0.221	0.396	0.671	0.617
OCT2 20% OAT3 10% OAT3 R4		0.058	0.037	0.377	0.527	1.00	0.904

OCT2 20% OAT3 10%		0.079	0.065	0.500	0.621	1.27	1.12
Sim GFR less than 30		0.396	0.670	20.3	11.5	32.9	31.8
OCT2 20% OAT3 10% OCT2 R2 OAT3 R4		0.065	0.055	2.98	3.34	6.44	6.32
OCT2 20% OAT3 10% OCT2 R2	25	0.039	0.028	3.52	3.82	7.41	7.34
OCT2 20% OCT2 R2 OAT3 R4		0.009	0.000	4.19	4.37	8.57	8.56
OCT2 20% OAT3 10% OCT2 R2 OAT3 R4 OATP1B3 R1		0.452	0.308	2.98	4.85	8.59	7.83
OCT2 20% OAT3 10% OCT2 R2 OATP1B3 R1		0.413	0.289	3.52	5.46	9.69	8.98

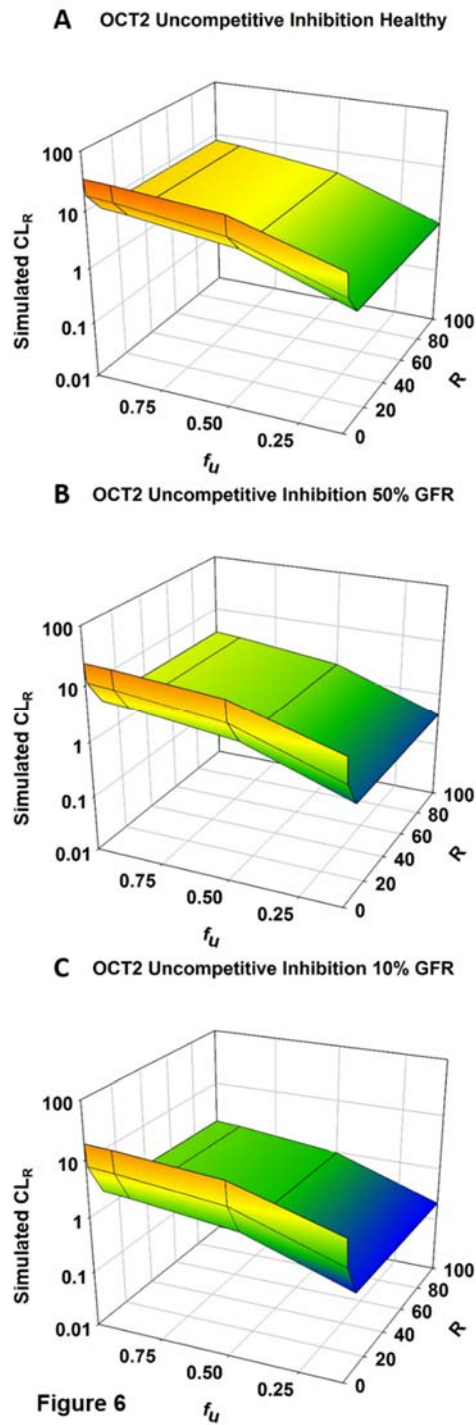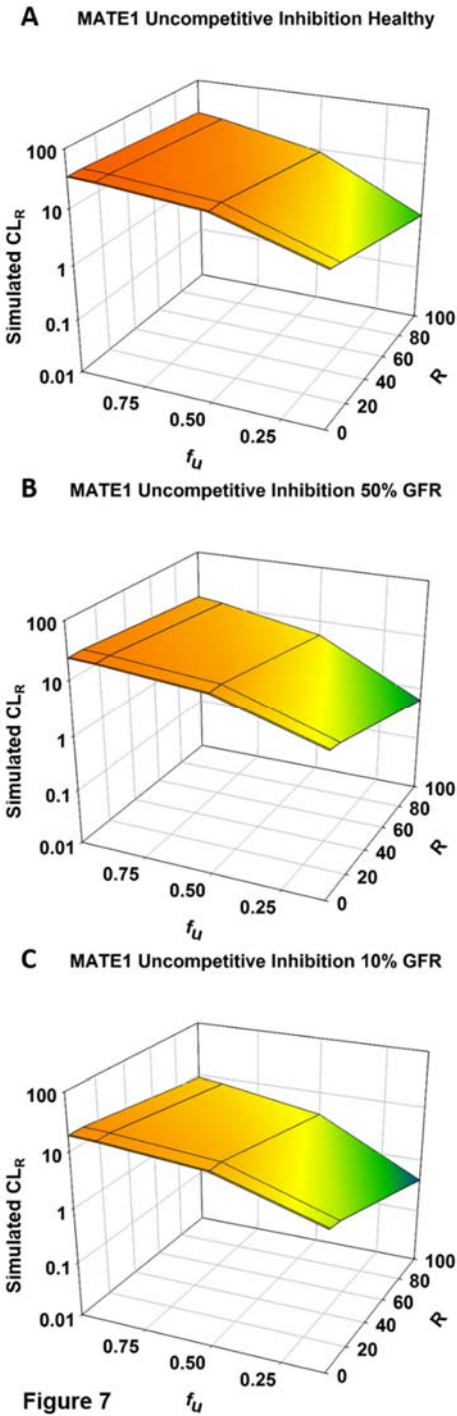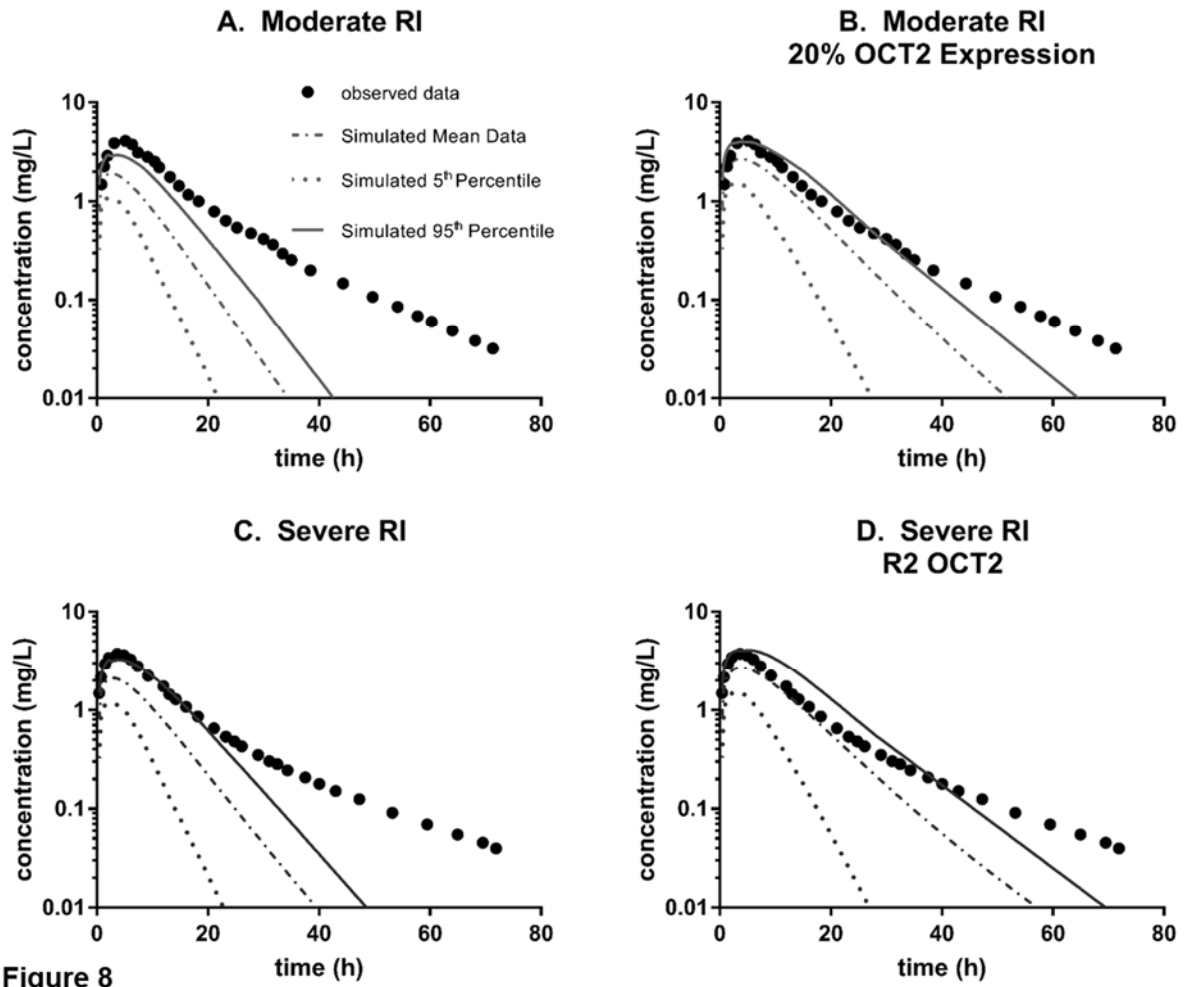**Figures:**

Figure 1.
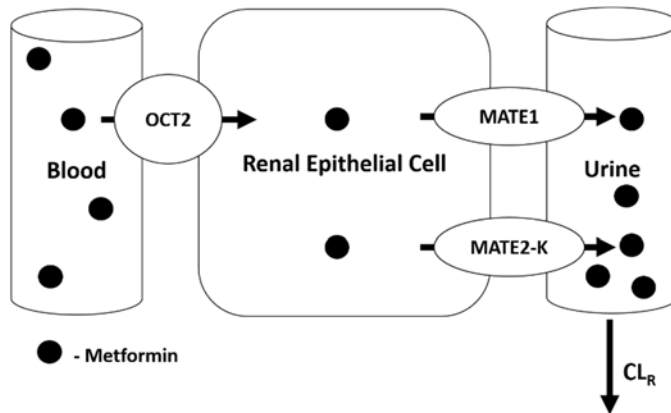
Figure 1

Figure 2.

Figure 2

Figure 3.

Figure 3

Figure 4.

A. Non-Competitive Inhibition OCT2

B. Non-Competitive Inhibition MATE1
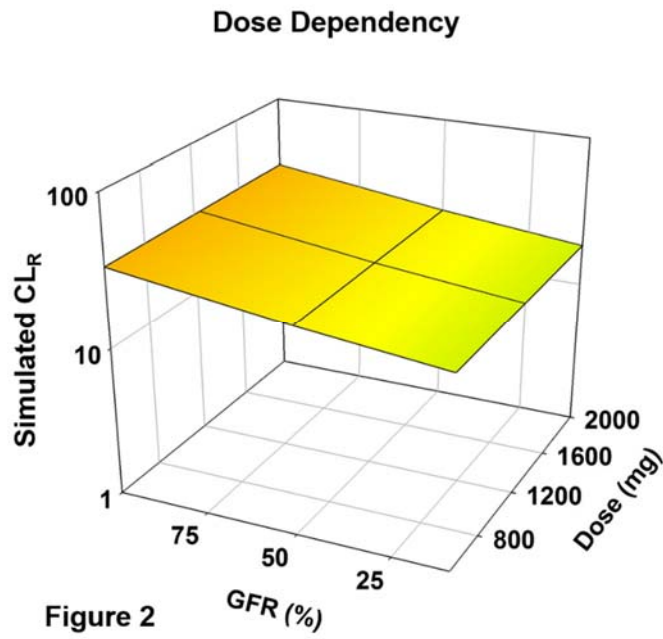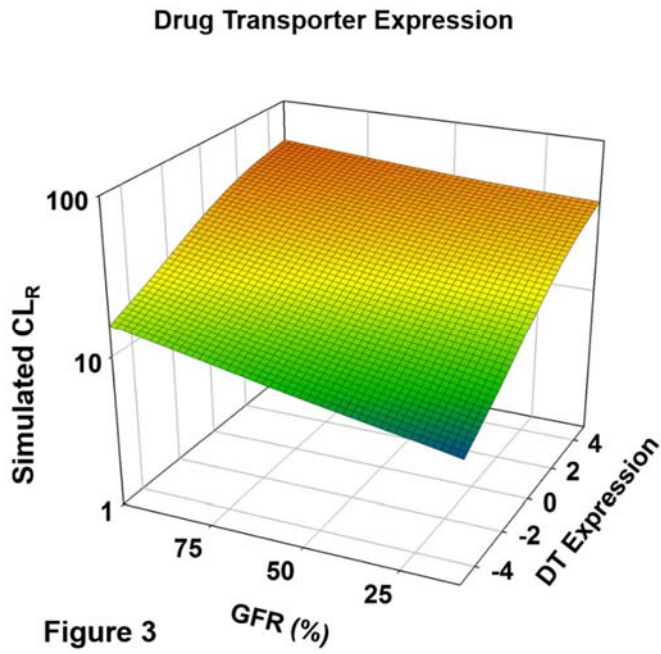
Figure 4

Figure 5.

Figure 6.

A OCT2 Uncompetitive Inhibition Healthy

B OCT2 Uncompetitive Inhibition 50% GFR

C OCT2 Uncompetitive Inhibition 10% GFR

Figure 6

Figure 7.

A MATE1 Uncompetitive Inhibition Healthy

B MATE1 Uncompetitive Inhibition 50% GFR
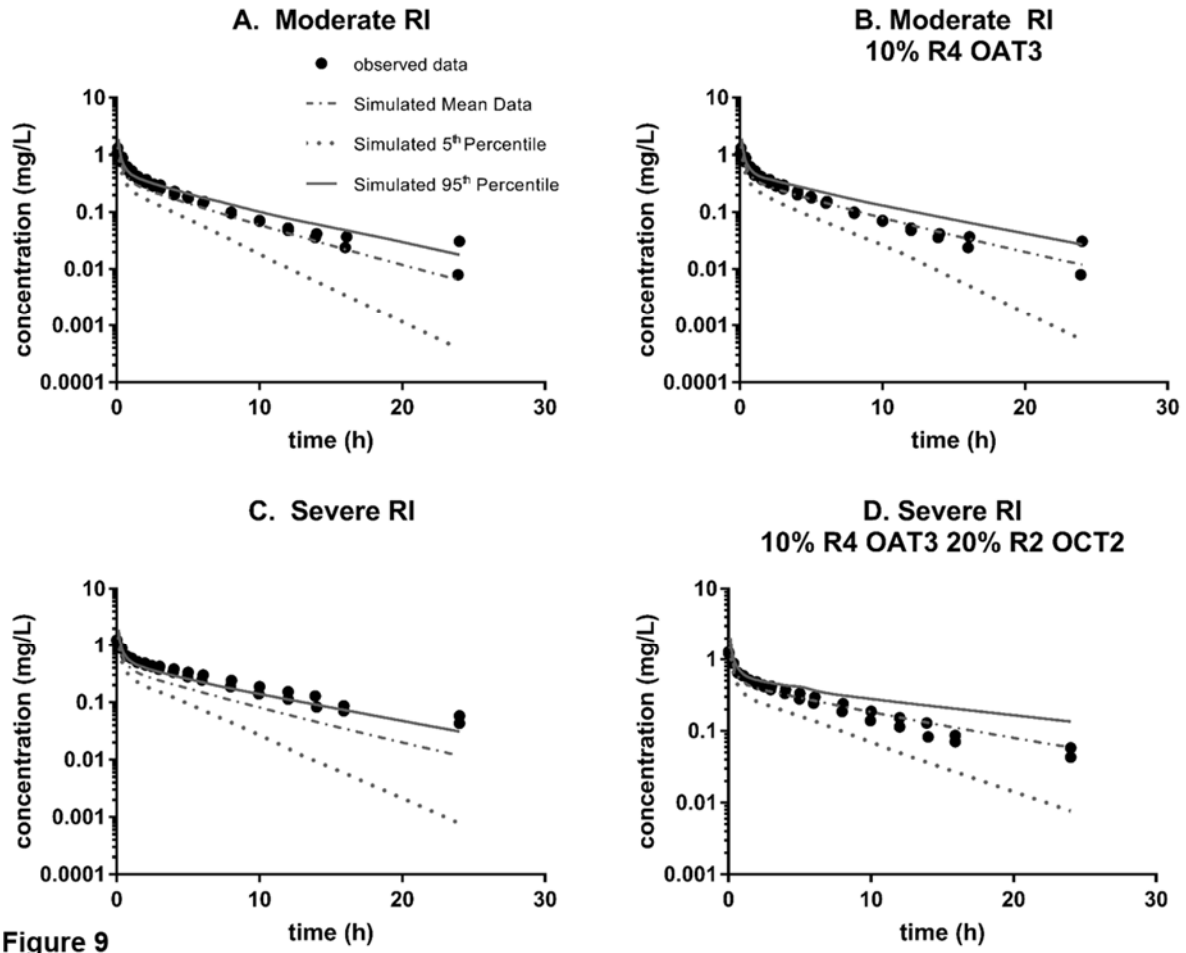

C MATE1 Uncompetitive Inhibition 10% GFR
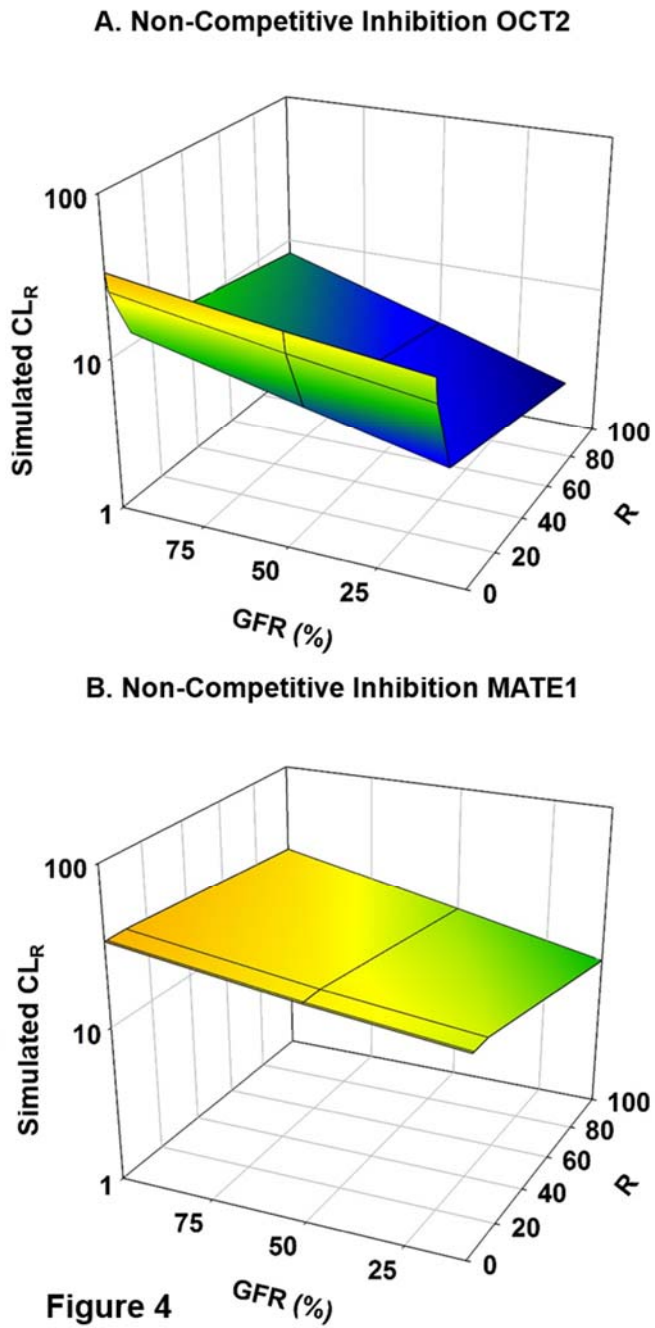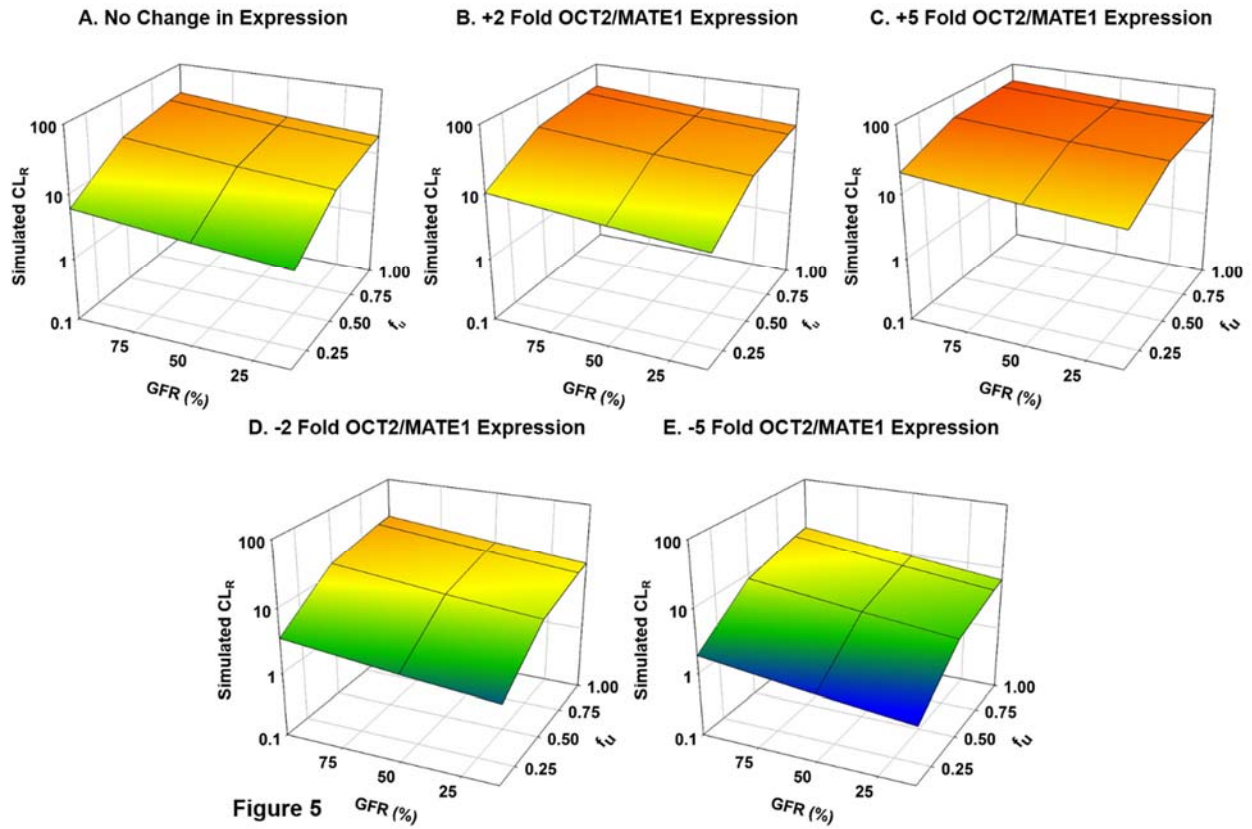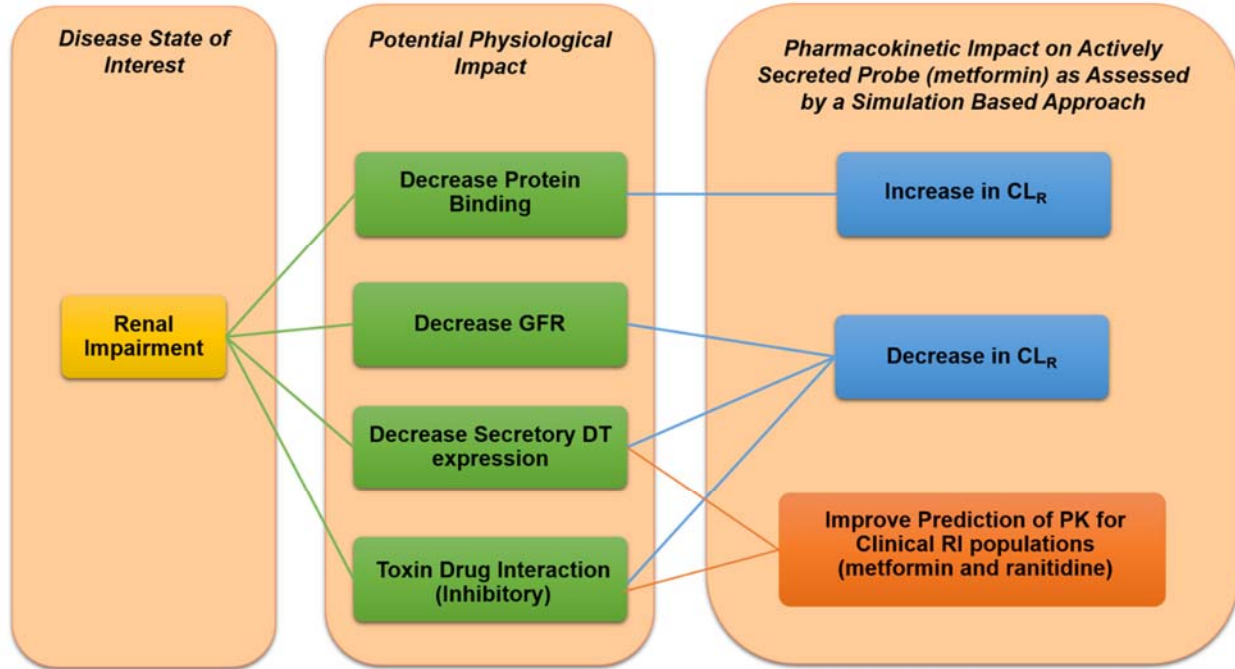

Figure 7

Figure 8.

Figure 8

Figure 9.

Figure 9

Figure 10.

Figure 10