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Using a semi-mechanistic model to identify the main sources of variability of metformin pharmacokinetics

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Abstract

Metformin pharmacokinetics (PK) is highly variable and researchers have for years tried to shed light on determinants of inter-individual (IIV) and inter-occasion variability (IOV) of metformin PK. We set out to identify the main sources of PK variability using a semi-mechanistic model. We assessed the influence of subject characteristics, including seven genetic variants. Data from three studies of healthy subjects with PK measurements of plasma and urine after single dose or at steady-state were used in this study. In total, 87 subjects were included (16 cross-over subjects). Single nucleotide polymorphisms in *ATM*, *OCT1*, *OCT2*, *MATE1* and *MATE2-K* were investigated as dominant, recessive or additive.

A 3-compartment model with transit absorption, renal elimination with a proportional error was fitted to the data using NONMEM 7.3. Oral parameters were separated from disposition parameters as dose-dependent absolute bioavailability was determined with support from urine data. Clearance was expressed as net renal secretion and filtration, assuming full fraction unbound and fraction excreted. Mean transit time and peripheral volume of distribution were identified as the main sources of variability according to estimated, with 94% IOV and 95% IIV, respectively. Clearance contributed only with 16% IIV. Glomerular filtration rate and body weight were the only covariates found to affect metformin net secretion; reducing IIV to 14%. None of the genetic variants were found to affect metformin PK.

Based on our analysis, finding covariates explaining absorption of metformin is much more valuable in understanding variability and avoiding toxicity than elimination.

Background

Metformin is an oral glucose-lowering drug used in the treatment of type 2 diabetes (T2D). Metformin is the first-line treatment due to its safety profile [1] and proven effect on diabetes-related morbidity and mortality [2]. While being efficient for lowering glucose and decreasing mortality, it is not sufficient as monotherapy in 15-30% of patients with T2D [3,4]. The main safety concern for metformin is an elevated risk of lactic acidosis, which has been shown to be loosely or unrelated to metformin plasma concentrations above 5 mg/l [5-7].

Finding predictive markers of variability would aid tailoring metformin therapy and potentially improve metformin efficacy and safety. At physiological pH metformin is protonated and relies on transporters to cross membranes. Metformin is a substrate to several groups of transporters: organic cation transporters (*OCT*) [8], multidrug and toxin extrusion transporters (*MATE*) [9], plasma monoamine transporters [10] and choline and serine transporters [11]. Genetic variations of abundance and function of these transporters may affect absorption, distribution and elimination of metformin.

The role of genetic variation has been investigated and even though metformin pharmacokinetics (PK) varies substantially [12], it does not appear to be tightly genetically controlled [13]. There has been a large number of clinical studies investigating the influence of specific single nucleotide polymorphisms (SNPs) on the metformin PK and pharmacodynamics (PD) but a number of negative findings [14,15] indicate that implementing metformin pharmacogenetics might be more complicated than first assumed and thus, the initial hype has been replaced by scepticism.

Population modelling efficiently makes use of data with few subjects and maintained statistical [16,17]. Thus, several population metformin PK models have been developed with the aim to identify covariates, e.g. weight, kidney function and genetic polymorphisms [18–24]. Body weight and estimated glomerular filtration rate (eGFR; a kidney function measurement) are covariates of oral volume of distribution and oral clearance, respectively, in approximately two thirds of the models. Non-renal clearance was investigated in two models, but a physiological separation of renal elimination on filtration, secretion and reabsorption has not previously been investigated. Presence and impact of SNP vary considerably between models, depending on available measurements and statistical power to discriminate covariate relationships. All published analyses assessed covariates on oral parameters, limiting the possibility to conclude which source of variability was reduced, absorption or elimination/disposition.

In this study, we combined data from three clinical studies where metformin plasma and urine was measured in order to develop a semi-mechanistic model, separating absorption from disposition parameters. By acknowledging the PK mechanisms and separating absorption from disposition, we hope to be able to identify the sources of PK variability. To try to explain the unexplained variability, we investigated the impact of seven known genetic variants and subject characteristics on metformin PK using the developed semi-mechanistic model.

Material

Data

Data came from three clinical studies conducted at Clinical Pharmacology and Pharmacy, Department of Public Health, University of Southern Denmark (clinicaltrials.gov identifier: NCT01726764, NCT01400191, NCT01237522) [15, 25, 26]. All studies included adult healthy subjects, receiving metformin either as single dose (study 3[26]) or multiple dose until steady-state at day 7 (study 1 and study 2[15,25]). Blood was collected for metformin concentration determination pre-dose and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5 (not study 1), 6, 7 (only study 2), 8, 10, 12 and 24 hr (not study 2) and urine was collected 12 and 24 hr (not study 2) after only/last dose. In total 87 subjects were included in the studies: 34 subjects in single dose study (study 3), 37 in steady-state studies (study 1 or 2) and 16 in both single and steady-state studies. The two steady-state studies had the same design: metformin was doses twice daily: first three 500-mg doses, followed by 1000-mg doses until day 7 when the PK sampling was performed. Body weight, age and eGFR (calculated using the Cockcroft-Gault formula with serum creatinine, age, weight and gender) were available at baseline for all subjects. Also, information about SNPs with reduced function of *OCT1* (rs12208357, rs34130495 rs72552763 and rs34059508) and SNPs in *OCT1*(rs622342), *OCT2* (rs316019), *MATE1* (rs2289669 and rs12943590), *MATE2-K* (rs12943590) and ataxia telangiectasia mutated (*ATM*) locus (rs11212617) was available in all studies. Information about *ATM* (rs11212617) was, however, missing for 41 and 8 subjects in the single dose study and study 2, respectively. *MATE2-K* (rs12943590) was missing for 34 subjects in the single dose study. An overview of the demographics is available in **Table 1**. All studies were approved by ethical and medical committees. Nominal time points were recorded for dosing and sampling for steady-state studies, while actual dosing and sampling times were recorded in the single dose study.

Analytical method for determination of metformin

Plasma and urine concentration of metformin was analysed by a high-pressure liquid chromatography method, which has previously been described in detail [27]. Briefly, the lower limit of quantification was 5 µg/l and 30 µg/l in plasma and urine, respectively, with a low inter- (plasma: 7.5% and urine: 6.2%) and intraday (plasma: 5.3% and urine: 1.8%) coefficients of variation. All plasma concentrations from the studies were above the lower limit of quantification.

Method

Model development and discrimination

Population modelling (also known as mixed-effects modelling) was used to estimate the parameters that, given the structure of the model, gives the best fit to the data, determined by maximizing the likelihood. This was done using the software NONMEM version 7.3 [28]. In population modelling, a parameter is described by two values: the fixed effect, or typical value, describing the central trend of the population and the random effect, describing the inter-individual (IIV) and/or inter-occasion (IOV) variability. The estimates of a parameters are the typical value of the population and the variance of the distribution that the population's random effects belong to. Thus, independent of how many individuals a population consists of, a parameter is described by these two estimates and thus the power increases largely with the approach compared to fitting the model to data of each individual, rendering as many parameters as there are individuals in the population.

Model discrimination was based on the likelihood ratio test (LRT, $p \leq 0.05$), goodness-of-fit graphics (e.g. observations versus population and individual predictions), and simulation properties through visual predictive checks (VPCs) [29]. The LRT states that the difference in likelihood between two hierarchical models is χ^2-distributed, and thus a drop in objective function value (OFV) ≥ 3.84 between models differing by one parameter being significant. Instead of assuming χ^2-distribution, the true critical value was determined through randomization tests with 500 samples for covariate modelling [30]. The software NONMEM was also used to perform the clinical trial simulations.

Structural model

Firstly, the structural model was investigated, testing one-, two- and three-compartment models. For absorption PK, three different models were considered: 1st order, transit compartment (1-5 transit compartments) and non-linear Michaelis-Menten kinetics, with saturable influx of metformin. Following mechanistic reasoning, eGFR was implemented as part of the structural model for filtration clearance and only the net secretion clearance (secretion minus reabsorption) was estimated (**Eq. 1-2**). As metformin is known to be actively excreted to a larger extent than reabsorbed, a positive value for this parameter was expected. The model, however, allowed for negative estimates, as would be with a net reabsorption. The fraction unbound (f_u) was assumed to be 1 [31].

$$CL_{net\ secr} = CL_{secr} - CL_{reabs} \quad (1)$$

$$CL_{tot} = eGFR \cdot f_u + CL_{net\ sec} \quad (2)$$

Deviations from nominal time of the penultimate dose (study 1 and 2) affects the pre-dose sample at day 7, thus also the residual error. To account for this, the nominal dosing time was used, however, estimating a lag-time of the penultimate dose, representing deviations from nominal time. A dose-dependent bioavailability has been reported for metformin [31] and thus the absolute bioavailability was allowed to vary between the two dose levels to reflect saturable absorption, assuming no metabolism of metformin (fe = 1) [31].

Stochastic model

The IIV was tested on all parameters in the model, assuming a log-normal distribution of random effects, except for IIV on bioavailability which was additive on logit scale. Correlations between IIVs were investigated between parameters of elimination and disposition and between parameters of absorption. Correlations of IIVs were, however, not investigated between elimination/disposition and absorption. Furthermore, to account for the 16 subjects present in both study 2 and 3, IOV was tested on all absorption parameters, using the same parameterization as for IIV. Additive, proportional or combined residual error structures were investigated.

Covariate model

To decrease run-times, the model was linearized before the stepwise covariate search [32]. For addition of a covariate into the model, $p \leq 0.05$ and $p \leq 0.01$ in the forward and backwards steps, respectively, were used. Three different models were used for testing effects of the seven genotypes: dominant (wt/wt *versus* variants), recessive (wt/wt and wt/v *versus* v/v) and additive (wt/wt *versus* wt/v *versus* v/v) on the PK parameters. All covariates were not tested on all PK parameters, instead a selection of relationships based on knowledge of transporter distribution in the body and their hypothesised effect on PK processes was done prior to testing. Age and body weight were investigated on the parameters as continuous covariates.

Lastly, eGFR was tested on net secretion clearance to investigate the impact of renal impairment on secretion. This was in addition to eGFR already being a part of the structural model for total clearance (Eq. 2).

The three different models of genotype effects were modelled as follows:

$$\theta_i = \theta_{TV} \cdot e^{\eta_i} \cdot \left(1 + \theta_{Gen} \cdot (Gen - \overline{Gen})\right) \quad (3)$$

And continuous covariates were modelled as follow:

$$\theta_i = \theta_{TV} * e^{\eta_i} * \left(1 + \theta_{cov} * (COV - \overline{COV})\right) \quad (4)$$

where θ_i is the subject's parameter estimate, θ_{TV} is the typical value for the population, η_i is the subject's random variability term, θ_{Gen} is the genotype effect size and $\overline{Gen}$ is the mean genotype value, θ_{COV} is the continuous covariate effect size and $\overline{COV}$ is the mean covariate value. For wt/wt, $\overline{Gen}$ was always set to 0. For wt/v, $\overline{Gen}$ was set to 1 for dominant and additive and 0 for recessive. For v/v, $\overline{Gen}$ was set to 1 for dominant and recessive and 2 for additive.

The covariates identified in the linearized covariate search were implemented in the non-linear model and tested for significance again.

Missing data

The *ATM* (rs11212617) and *MATE2-K* (rs12943590) were not determined for all subjects, only 52% were complete for both SNPs. Five different ways of handling the missing genotype data were assessed: (i) excluding incomplete cases, (ii) treating the missing genotype as wt/wt, (iii) wt/v, (iv) v/v, or (v) model-based estimation of most likely genotype [33]. In approach (v), the most likely variant was estimated using the background frequency of *ATM* (rs11212617) and *MATE2-K* (r12943590) (**Table 1**).

Simulations

Eight simulation scenarios (n=1000) were performed to investigate the impact of kidney function (scenarios 1-4) and explaining variability (scenarios 5-8) on C_{max}, and area under the curve (AUC). In scenarios 1-4, the maximum Standard-of-Care dosing was simulated for various degree of kidney impairment:

Patients with eGFR

1) 30 ml/min/1.73m^2: 500 mg twice daily
2) 45 ml/min/1.73m^2: 1000 mg twice daily
3) 60 ml/min/1.73m^2: 1000 mg trice daily
4) 90 ml/min/1.73m^2: 850 mg four times

Scenarios 1-3 are extrapolations outside the observed eGFR in the current study (Table 1). Weight was assumed to be higher than for healthy subjects, i.e. 87 kg. The equation by Bardin *et al.* [21], i.e. $F=(dose/780)^{-0.23}$, combined with the estimated absolute bioavailability was used to predict absolute bioavailability of 850 mg. C_{max} for each scenario was compared with the limit of toxicity, 5 mg/l.

Based on the results of scenario 1-4, the scenario with the highest risk of overexposing subjects to metformin was used as basis for scenario 5-8. Scenario 5-8 illustrated the benefit on AUC and C_{max} when explaining all variability in those two parameters with the highest estimated variability as well as total clearance and absolute bioavailability if these were not the two parameters with the highest variability.

Results

A total of 1,462 metformin plasma and 171 urine concentrations were obtained from 87 subjects (24 women and 63 men); 16 subjects had both single- and repeated dose plasma and urine concentrations. Demographics of included subjects are shown in **Table 1**.

Base model

A 3-compartment model with one transit compartment for absorption, first order elimination and a proportional error model best described the data. An overview of the model is shown in **Fig. 1**. The model was implemented in terms of absorption rate constant (k_a), mean transit time (MTT), absolute bioavailability (F), net renal secretion ($CL_{net\ secr}$), volumes of distributions: central (V_c) and peripheral (V_{p1} and V_{p2}) and inter-compartmental clearances (Q_1 and Q_2). Absolute bioavailability was estimated to 53% and 43% of 500 mg and 1000 mg metformin, respectively, with bioavailability of 500 mg being more precisely estimated than 1000 mg (3% and 6%, respectively). The IIV was initially added on all parameters. However, estimated uncertainty of IIV on parameters of the second peripheral compartment were high and thus IIV was removed from Q_2 and V_{p2}. Inter-occasion variability was added to bioavailability and mean transit time. With IOV in the model, the IIV on mean transit time was not statistically significant and thus excluded. Also, the correlation of IIV between inter-compartmental clearance and peripheral volume of distribution was close to 1 and thus fixed to full

correlation with different, estimated magnitudes. All parameters were precisely estimated as seen in **Table 2**. Since there was a high shrinkage of empirical Bayes estimates (range: IIV 7.2-29% and IOV 36-52%), discrimination was mainly guided by difference in OFV and VPCs. The visual predictive check for the base model is shown in **Fig. 2**.

Covariate models

The model was successfully linearized which facilitated the covariate search. Based on the final base model, the following relationships were selected for testing:

- *OCT1* (rs622342 and reduced function alleles) on k_a, F, $CL_{net\ secr}$, Q_1 and V_c
- *OCT2* (rs316019) on F, $CL_{net\ secr}$, Q_1 and V_c
- *ATM* (rs11212617) on k_a, F, $CL_{net\ secr}$, Q_1 and V_c
- *MATE1* (rs2289669 and rs2252281) on F, $CL_{net\ secr,}$ Q_1 and V_c
- *MATE2-K* (rs12943590) on F, $CL_{net\ secr}$ and Q_1

All tested covariates were time-invariant. Thus, as mean transit time was associated with IOV, no covariate testing was performed on this parameter. The full correlation between IIV on peripheral volume of distribution and inter-compartmental clearance implies that covariate testing on inter-compartmental clearance effectively tests covariates on peripheral volume of distribution. When investigating covariates with a two-compartment model, the results were the same.

Missing data was not randomly distributed between the studies and approach (i) removed > 80% of the subjects in the 500-mg single-dose study, affecting mainly the estimates of uncertainty to become larger. The non-random exclusion of data seems to affect estimates of 1000-mg bioavailability; typical individual, IIV and IOV decreased 18%, 48% and 17%, respectively (see **Table S1** of estimates in appendix). There were mostly small differences in disposition parameter estimates, with the exception of inter-compartmental clearance and peripheral volume of distribution, for which the typical individual estimate decreased by 26% and 27%, respectively. As the uncertainty of the parameter estimates increase with removal of data, the confidence intervals of the parameters overlap; thus, none of the mentioned trends above are statistically significant. In all five approaches for treating missing covariates, body weight was significantly included on net secretion clearance, but for approaches (ii-v) also eGFR on net secretion clearance entered the model. The addition of covariates on net secretion clearance reduced IIV from 16% to 14%; a minor improvement. All parameter estimates with uncertainties for the final model estimated on all data are shown in **Table 2** and for approach (i) in **Table S1** (appendix). **Fig. S2** in appendix shows the impact of covariates on net

secretion clearance as well as the PK profiles as function of maximum and minimum body weight and eGFR.

Simulations

The impact of kidney function and explaining variability of parameters on C_{max} and AUC at steady-state metformin PK is shown in **Fig. 3**. Setting absolute bioavailability of 780 mg to 46.8% in the equation by Bardin *et al.* [21], $F=0.466\ (Dose/780)^{-0.23}$, predicted an absolute bioavailability of 52% and 44% of 500 mg and 1000 mg metformin, respectively. The predicted bioavailability was of 850 mg was 45.9%.

The risk of over-exposure of metformin was low. C_{max} was predicted > 5 mg/l in only 0%, 1%, 3.2% and 0.4% of patients with eGFR 30, 45, 60 and 90 ml/min/1.73m^2 on the maximum dose according to Standard-of-Care, respectively. The patient group with eGFR between 89 and 60 ml/min/1.73m^2 had the highest risk of over-exposure, and thus, this scenario was used as reference for simulations of scenarios 5-8. When looking at overall exposure, i.e. AUC as opposed to C_{max}, the patient group with the exposure was eGFR between 59 and 45 ml/min/1.73m^2.

Mean transit time and peripheral volume of distribution had the largest inter-occasion (IOV=94%) and inter-individual (IIV=95%) variability, respectively, and was therefore investigated in the simulations of effects of fully explaining variability. C_{max} was predicted > 5 mg/l in 2.2%, 2.9%, 0% and 2.8% of patients if covariates were assumed to explain all variability in clearance, peripheral volume of distribution, mean transit time and bioavailability, respectively. Thus, although explaining variability in clearance reduced the risk from 3.2% to 2.2%, explaining variability in mean transit time was more beneficial, predicting 0% risk of over-exposure.

Discussion

We present a semi-mechanistic population model of metformin PK, separating the renal net secretion from the renal filtration, determining the absolute dose-dependent bioavailability and by that separating absorption from disposition. In doing so, the estimates of variability in the model suggested that mean transit time and peripheral volume of distribution were the PK parameters with the highest variability and that total clearance with eGFR carries little of the variability. Upon simulations,

explaining the variability of mean transit time was shown to have the greatest effect on reducing variability in C_{max}. A thorough covariate search was performed and two significant covariates were identified, although none of the investigated genetic variants affected metformin PK. Body weight and eGFR affected the renal net secretion of metformin, which changed from 16% variability to 14%, a minor improvement. No covariates were investigated for mean transit time as this parameter was associated with inter-occasion variability and all available covariates were time-invariant.

For the simulations, metformin PK was assumed to be the same for healthy subjects and patients with T2D, with the only difference being difference in weight. Goswami *et al.* [18] analysed PK data from patients with T2D and healthy subjects and found no difference in PK, although weight was a covariate in their model. The healthy population investigated in this paper invalidates any attempts to investigate clinical impact though, as PD is different in healthy subjects compared to patients with T2D.

Several structural models for metformin PK have previously been proposed; both one- [19,21,22,24] and two-compartment models [18,20,23]. The discrepancy between reported structures is likely related to the duration of the blood sampling, with one-compartment models being reported in studies where the final blood sample was drawn < 15 hr post-dose [21,22,24], and two-compartment models being reported when data included samples up to 24 hr post- dose [18,20,23]. Failure to identify the second phase of the metformin PK will impact AUC extrapolations and predictions of steady-state concentrations, as the accumulation of metformin is underestimated. The 3rd compartment identified in the current study was associated with a drop in OFV of 12.9. Although statistically significant, simulations of metformin steady-state concentrations showed small differences between two- and three-compartment models (data not shown) and thus the clinical importance of this third compartment is minor, unless drug distribution can be linked to effect site concentrations.

Although absolute bioavailability was estimated, there was no support for a continuous absorption model with only two doses. Bioavailability for 1000 mg was lower than for 500 mg (43% compared to 53%), most likely due to saturable absorption. Bioavailability has previously been reported to vary from 40-60%, which is in line with our findings [30]. Using the model developed for relative bioavailability by Bardin *et al.* [21], correcting for the estimated bioavailability, predicted a deviance of less than 2.5% for the two doses. Thus, it seems that within the range investigated in this study, Bardin's approximation works well.

Several covariates have previously been reported to affect metformin PK. Considering that metformin is eliminated primarily through the kidneys, it is unsurprising that kidney function is predictive of metformin clearance in most metformin PK models [18,20–22,24]. Due to this, we included kidney function in the structural model. When thereafter testing the importance of eGFR on net secretion clearance, a OFV drop of 9.4 was observed. This indicates that eGFR is a measurement of kidney function in general and not only the filtration, as filtration effects were already accounted for through $CL = eGFR \cdot fu + CL_{net\ secr}$. According to our findings, eGFR is responsible for around 21-25% of total clearance in the range of eGFR investigated (70-122 ml/min/1.73m^2) and thus the effects on steady-state concentrations is moderate, similar to the conclusion reached by Duong *et al.* [20]. The main difference between our approach and the approach used by Duong *et al.* is that we allow for different impact of eGFR on filtration and net secretion.

The simulations of maximum Standard-of-Care treatment with eGFR of 30 ml/min/1.73m^2 showed that the steady-state PK of metformin was well below the toxicity limit, with no patients having C_{max} > 5 mg/l. This is in line with recent findings by Lalau *et al.* [34] where metformin was found to be safe and efficacious in moderate to severe kidney disease. Patients with low eGFR may suffer from a range of systemic complications simultaneously, and metformin accumulation is probably not the cause of lactic acidosis in these subjects in most cases [35, 36]. This is also supported by the work by Aharaz *et al.* [7] who found no relation between metformin and neither the incidence rate of or risk of acute hospitalization with acute lactic acidosis. Notable is, however, that the most extreme subjects with moderate to severe (45-59 ml/min/1.73m^2) and mild to moderate (60-89 ml/min/1.73m^2) kidney impairment were according to the simulations at risk of overexposure. Thus, there may be a higher chance of finding a relationship between metformin exposure and lactic acidosis from epidemiological studies in these groups, as opposed to the most extreme group as the exposure is higher. To couple this PK model with a model describing the lactic acid concentrations as a function of metformin concentrations would enable a quantitative assessment of relationship. The results should, however, be regarded with care as the lowest eGFR studied here was 70 ml/min/1.73m^2.

Finding covariates to explain PK variability for a better metformin dosing has been a focus of metformin PK research for long [12,14,15,18-26,36] and clearance has previously been reported to be highly variable [18,20-22,24]. However, in our study, the variability was only 16%. This may be due to separation of variability on absorption and elimination, in the current work. According to our results, there is thus more to gain in investigating covariates explaining the absorption processes than elimination processes. SNPs of *OCT*-1, *OCT*-2 and *MATE2-K* would potentially affect bioavailability,

however, no correlation was identified in this work. *OCT-3* is known to be located in the intestine and thus SNPs of this transporter may affect bioavailability as well [37]. However, as this was unavailable in the current study, it was not tested. There was a large proportion of IOV for mean transit time where genetic variations cannot explain the difference.

Similar to previously published population metformin PK modelling by Duong *et al.* [20], no genotypes were included as covariates in this model. Two of the SNPs investigated in this work have previously been identified as significant for inter-compartmental clearance and central volume of distribution by Goswami *et al.* [18]. The failure to identify SNPs on the PK of metformin in the current model may be due to the few subjects compared to Goswami *et al.* (87 subjects compared to 546 subjects) or differences between PK of healthy volunteers and patients with T2D, although the latter seems unlikely. Notably, we do not believe that the failure to identify the covariate relationships is related to the distribution of SNPs in the studied population, which was adequate. A recent study reported on a correlation between a SNP close to *ATM* and metformin PK [38]. However, we could not identify such correlation.

In conclusion, we successfully developed a semi-mechanistic model describing metformin PK in healthy subjects, determining the absolute dose-dependent bioavailability, separating the disposition parameters from the absorption parameters and the clearance of metformin into renal filtration and net secretion. This study shows that there is more to gain by explaining the variability in absorption than elimination and that the identified covariates body weight and kidney function on metformin clearance had little effect on the overall PK of metformin.

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Table 1. Demographics of included subjects in the three studies. Mean and range (min-max) is given for continuous covariates and for genotypes, the proportions of wildtype (wt|wt)/heterozygote variant (wt|v)/homozygote variant (v|v) is given for the subjects with information about the SNP. BMI – body mass index; eGFR – estimated glomerular filtration rate; *OCT* – organic cation transporters; *MATE* – multidrug and toxin extrusion transporters; *ATM* – ataxia telangiectasia mutated locus.

Covariate	**All studies**	**Study 1 steady-state**	**Study 2 steady-state**	**Study 3 single dose**
No of subjects	87	19	34	50
Age [years]	26.6 (20-49)	25.4 (22-30)	27.3 (22-46)	26.1 (20-49)
Weight [kg]	76.6 (53-115)	78.8 (67-96)	75.3 (54-104)	77.0 (53-115)
BMI [kg/m^2]	23.8 (20-34)	24.0 (22-29)	23.7 (20-31)	23.8 (20-34)
eGFR [ml/min/1.73m^2]	96.2 (70-122)	95.1 (72-107)	96.0 (70-122)	97.3 (76-121)
OCT1 reduced alleles [%]	56/28/16	100/0/0	35/38/27	54/32/14
OCT1 (rs622342) [%]	42/41/17	53/47/0	36/32/32	42/44/14
OCT2 (rs316019) [%]	64/28/8	100/0/0	65/26/9/	50/40/10
MATE1 (rs2289669) [%]	32/46/22	47/21/32	23/59/18	32/46/22
MATE1 (rs12943590) [%]	28/52/20	16/53/31	23/62/15	36/44/20
MATE2-K (rs12943590) [%]	41/55/4	37/52/11	44/53/3	37/63/0[†]
ATM (rs11212617) [%]	37/48/15	47/47/6	35/42/23[‡]	23/66/11[§]

[†] 68% of all subjects in study 3 missing information about *MATE2-K*

[‡] 24% of all subjects in study 2missing information about *ATM*

[§] 82% of all subjects in study 3 missing information about *ATM*

Table 2. Table of estimates of the semi-mechanistic model for metformin pharmacokinetics using all available data. Relative standard error (RSE) and coefficient of variance (CV) are given in %. The CV expresses the inter-individual variability (IIV) or inter-occasion variability (IOV).

Parameter		**Typical value**	**IIV-CV**	**IOV-CV**
$CL_{net\ secr}$	Net secretion clearance [L/h]	25.5 (2.8)	14 (20)	
Body weight on $CL_{net\ secr}$ [%/kg]		0.610 (32)	-	-
eGFR effect on $CL_{net\ secr}$ [%/ml/min/1.73m^2]		0.484 (46)	-	-
V_c	Central volume of distribution [L]	38.5 (19)	36 (28)	
Q_1	Inter-compartmental clearance [L/h]	8.92 (12)	69 (12)	
V_{p1}	Peripheral volume of distribution [L]	347 (18)	93 (14)*	
Q_2	Inter-compartmental clearance [L/h]	14.8 (49)	-	
V_{p2}	Peripheral volume of distribution [L]	12.1 (33)	-	
MTT	Mean absorption transit time [h]	0.272 (25)	-	91 (17)
k_a	Absorption rate constant [h^{-1}]	0.322 (5.5)	11 (18)	
F_{500}	Bioavailability dose 500 mg [%]	52.5 (3.4)	21 (45)**	30 (29)**
F_{1000}	Bioavailability dose 1000 mg [%]	42.6 (5.6)		
$T_{pre\text{-}dose}$	Time since prior dose steady-state [h]	10.5 (20)	87 (22)	
$\sum_{plasma}$	Proportional residual error plasma [%]	11.8 (1.9)	-	
$\sum_{urine}$	Proportional residual error urine [%]	18.4 (8.9)	-	

* Full correlation between IIV of Q_1 and V_{p1}, estimating difference in magnitude to 1.35 times higher for V_{p1} with RSE=6.8%.

** Same parameter estimated for IIV and IOV of F_{500} and F_{1000}.

Figure 1. **Schematic representation of the semi-mechanistic model of metformin pharmacokinetics.** A three-compartment disposition model with one-compartment transit absorption and first order elimination described the pharmacokinetic metformin data well. The model was implemented in terms of absolute bioavailability (F), mean transit time (MTT), absorption rate constant (k_a), central and peripheral volumes (V_c, V_{p1} and V_{p2}), inter-compartmental clearances (Q_1 and Q_2) and clearance (CL_{tot}). Clearance was defined as the filtration and the net secretion clearance, assuming only renal elimination (fe = 1) and all drug being unbound (fu = 1).

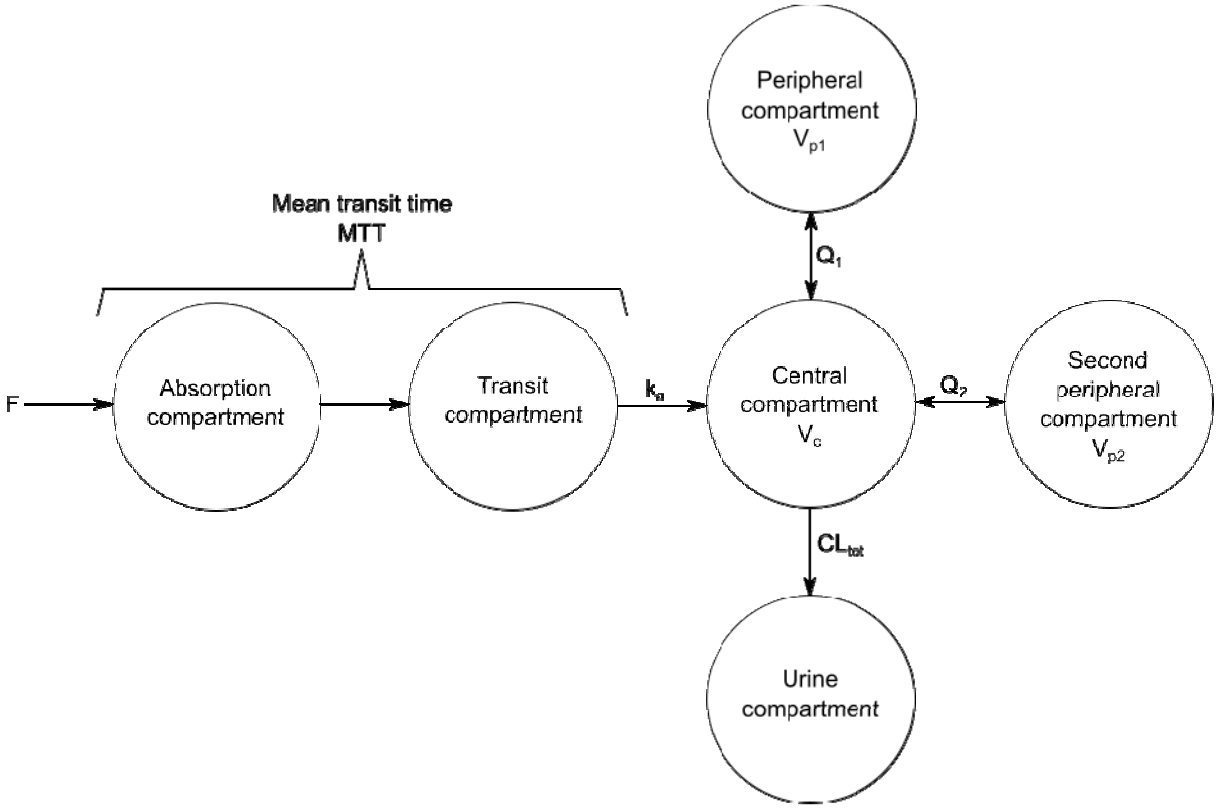

Figure 2. **Visual predictive check (VPC) of model fit.** The VPC shows the metformin plasma concentration (top) and urine amount (bottom) for single (left) and repeated (right) dose. The open circles represent the actual observed concentrations. The lines represent the median (solid), 5th and 95th (dotted) percentiles of the observed concentrations. The shaded areas represent the 95% confidence intervals of the predictions for the corresponding percentiles. This VPC shows the base model without covariates, however, there is no visible difference in the VPC with covariates.

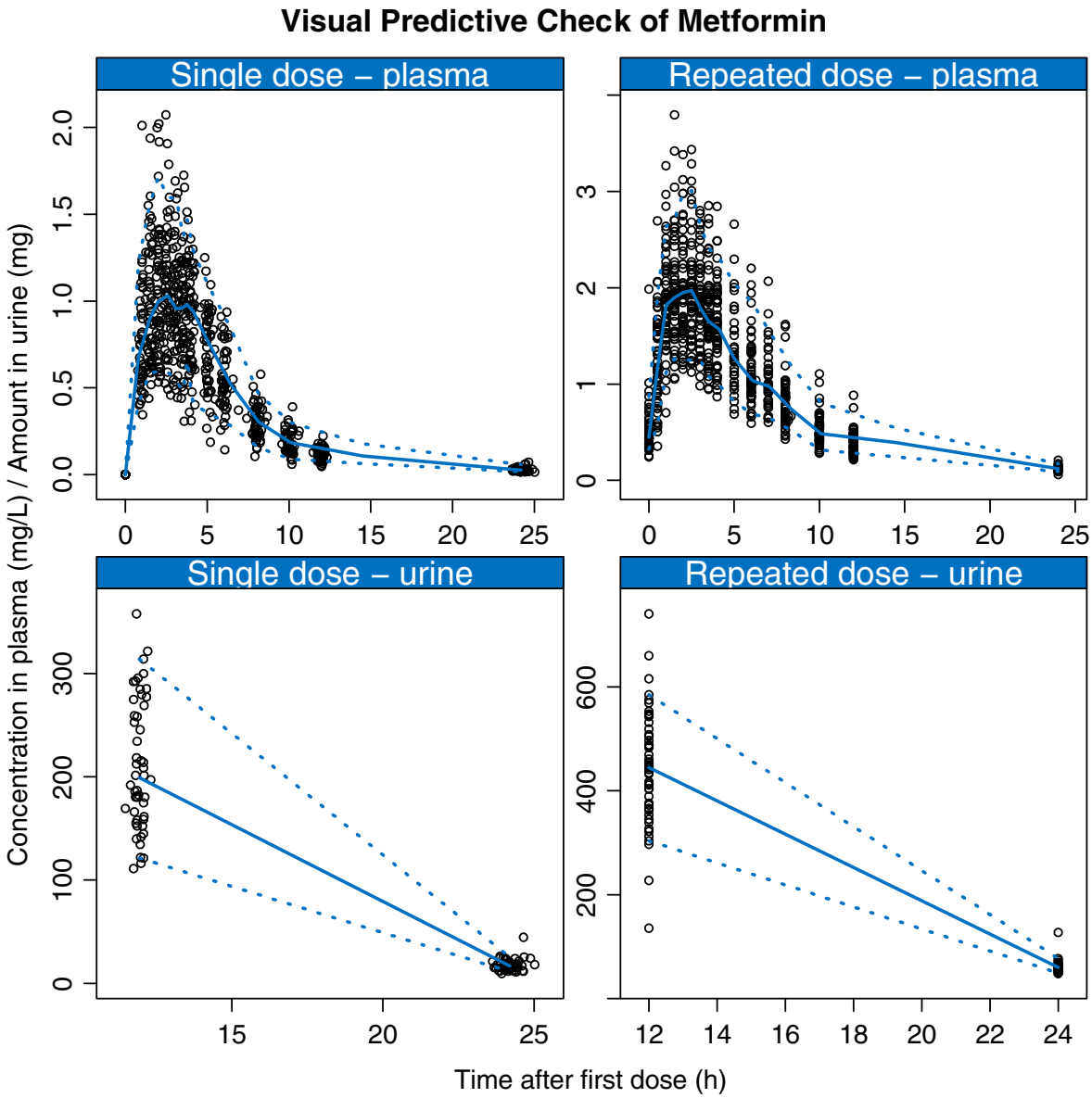

Figure 3. **Impact of covariates and variability on pharmacokinetic indices.** Thousand simulations were performed for eight scenarios: maximum standard of care dosing for patients with estimated glomerular filtration rate (eGFR) 30, 45, 60 and 90 ml/min/1.73m^2, as well as for patients with eGFR 60 ml/min/1.73m^2 without variability on clearance (CL_{tot}), peripheral volume of distribution (V_{p1}), mean transit time (MTT) and absolute bioavailability (F) to visualise the impact of the main covariate eGFR and the variability of parameters on exposure (area under the concentration-time curve, AUC) and maximum concentration (C_{max}). The highest investigated concentrations, and thus guiding concentration for clinic, has been indicated at 5 mg/l. Body weight was assumed to be higher than during model development to mimic a reasonable weight for patients with type 2 diabetes, i.e. 87 kg.