

Supplementary Materials

Virtual Twin-PBPK Modelling: A Step Toward Precision Dosing in Patients with Obesity

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1. Base model: Input parameters

In Step 0 of the VT-PBPK modelling workflow, the default input parameters provided in Simcyp V23 were used as the baseline for model development. This initial step employed the default Simcyp population settings to simulate both obese and morbidly obese individuals (Sim-Obese and Sim-Morbidly Obese). The purpose was to establish a population-level reference model for the two drugs under investigation, midazolam and digoxin, before applying any individual-specific adaptations in subsequent steps.

Supplementary Table 1 Input parameters for Step 0, default Simcyp V23

Parameter Category	Sim-Obese	Sim-Morbidly Obese	Reference (1)
Demographics			
Age, sex, height, weight, BMI	Adjusted to reflect population with obesity	Adjusted to reflect population with severe obesity	Based on the health survey for England database 2009-2018
Blood Composition			
Haematocrit (%)	M-43 (6.5) and F-38 (7.1)	M-43 (6.5) and F-38 (7.1)	Based on meta-analysis
Serum Albumin (g/L)	M-50.34 and F-49.38 (10)	M-50.34 and F-49.38 (10)	Based on meta-analysis
α 1-Acid Glycoprotein (AGP) (g/L)	Increased by ~32%	Increased by ~74%	Due to low-grade inflammation associated with obesity
Organ Blood Flows			
Cardiac Output (CO)	Proportional to BSA	higher BSA	Adjustments made to reflect physiological changes
Liver Blood Flow	Based on CO	Based on CO	Maintained as a percentage of CO
Kidney Blood Flow	Based on CO	Based on CO	Maintained as a percentage of CO
Adipose Tissue Blood Flow	Increased to reflect expanded adipose mass	Further increased to reflect greater adiposity	Proportional to adipose tissue volume

Parameter Category	Sim-Obese	Sim-Morbidly Obese	Reference (1)
Gastrointestinal (GI) Physiology			
Gastric Emptying Time	No significant change	No significant change	Based on available studies
Small Intestinal Transit Time (SITT)	No significant change	Slight increase observed	Not deemed clinically significant
Colonic Transit Time	Potential decrease	Potential decrease	Further data required
Renal Function			
Glomerular Filtration Rate (GFR)	Estimated using MDRD equation	Estimated using MDRD equation	Based on meta-analysis of 18 studies
Serum Creatinine	Age <61, 76.5 (16.1) Age >61, 81.2 (27.4)	Adjusted accordingly	Reflects changes in muscle mass and renal function
Enzyme and Transporter Expression			
Hepatic CYP3A4 ^a	114 (41%)	114 (41%)	Meta-analysis
Hepatic CYP3A5 ^a	Based on correlation with CYP3A4	Based on correlation with CYP3A4	Simcyp Simulator V23
Hepatic UGT1A4 ^a	52 (26%)	52 (26%)	Meta-analysis
Hepatic P-gp ^b	0.246 (59%)	0.246 (59%)	Meta-analysis
Intestinal CYP3A4 ^c	65.4 (52%)	65.4 (52%)	Meta-analysis
Intestinal CYP3A5 ^c	23.3 (32%)	23.3 (32%)	Meta-analysis
Intestinal P-gp ^b	0.4	0.4	Meta-analysis

^aExpressed as pmol/mg microsomal protein; ^b Expressed in pmol/millions of hepatocytes; ^cExpressed in nmol/small intestine; ^bExpressed as pmol/mg total membrane protein; BSA-Body Surface Area; MDRD-Modification of Diet in Renal Disease Equation.

2. Drug-specific parameters

Midazolam model was modified from the previously developed model in Simcyp Simulator v23 (Certara, UK) library. To transition from the minimal PBPK model available in the library to a full-body PBPK model that better accounts for altered tissue distribution with increased BMI, the tissue-to-plasma partition coefficient (K_p) scalar was optimized to improve the accuracy of the distribution phase. The optimization was initially performed using data from healthy individuals after both IV and oral administration of midazolam at different dose levels (2-4) and subsequently extrapolated to the population with obesity using an independent clinical dataset (5 mg IV and 10 mg oral) comprising 20 subjects with obesity (5). The Rodgers and Rowland method (6, 7) was applied to predict the volume of distribution (V_d).

The previously developed full-body PBPK model for digoxin in healthy individuals was based on established frameworks described in Neuheff et al. (8, 9) which provided comprehensive characterization of digoxin pharmacokinetics, including P-glycoprotein-mediated efflux. The ADAM absorption model was applied to capture the absorption phase of digoxin. Unlike midazolam, no further optimization of distribution parameters was performed for digoxin, as the default parameters (based on mechanistic predictions) adequately reproduced known pharmacokinetic profiles in healthy populations. Full Body PBPK model for digoxin in healthy individuals was used for simulations. The ADAM absorption model was applied to capture the absorption phase. Furthermore, no independent dataset for individuals with obesity was available for digoxin in literature to verify the model as was done for midazolam. The Rodgers and Rowland (6, 7) method was employed to predict the V_d for digoxin, ensuring consistency in the mechanistic approach across both drugs investigated.

Supplementary Table 2 Compound specific input parameters for VT-PBPK model of midazolam and digoxin

Model input parameters	Values*		ref
	Midazolam	Digoxin	
Molecular weight (g/mol)	325.8	780.94	
LogP	3.53	1.26	

pKa	6	~7.2
Compound type	Monoprotic base	Neutral
B/P ratio	0.603	1.07
Fraction unbound in plasma	0.032	0.71
Absorption	First order	ADAM Model
Distribution and elimination		
Distribution model	Full body PBPK	Full body PBPK
Vss prediction method	Method 2	Method 2 (6, 7)
Kp scalar	0.25 (optimised)	1

B/P ratio- blood to plasma ratio; Vss- Volume of distribution at steady-state; P-gp- P-glycoproteins
 * The compound-specific parameters are based on validated literature and internal datasets used by Certara were applied without modification unless otherwise stated.

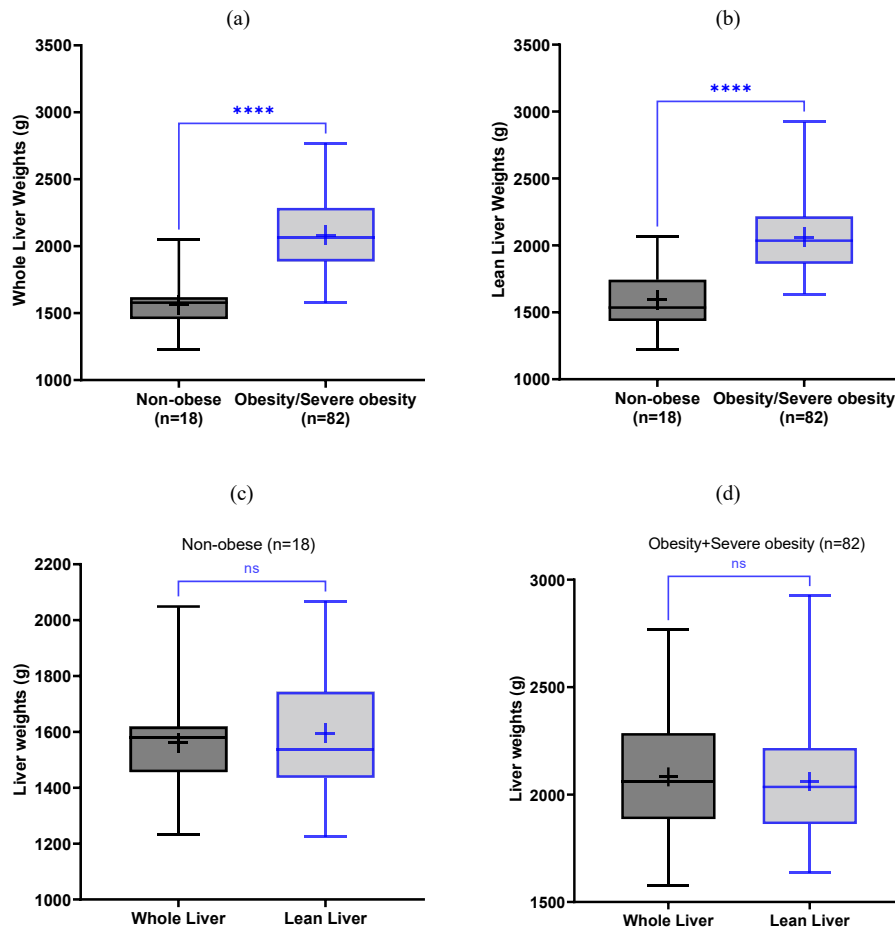
Supplementary Table 3: Base model predicted PK parameters

PK parameter	Mean Observed	Mean model predicted (Step 0)
Midazolam		
C _{max} , oral (ng/mL)	6.2	7.03
AUC _{0-inf,iv} (ng*h/mL)	60.9	41.44
AUC _{0-inf,oral} (ng*h/mL)	12.1	16.3
Digoxin		
C _{max} (ng/mL)	1.72	2.1
AUC ₀₋₂₄ (ng*h/mL)	11.7	15.4

3. Dosing and blood sample collection information

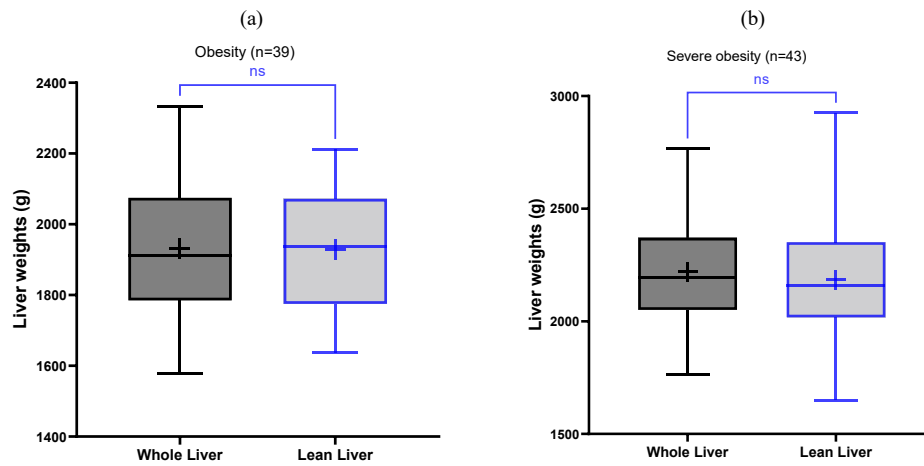
The drug cocktail is administered with water on the day of PK investigation in fasting condition. The COCKTAIL includes losartan tablets (2x12.5 mg), omeprazole enterotablets (1x20 mg), digoxin tablet (0.5 mg), midazolam oral syrup (1.5 mg) followed by intravenous midazolam (1.0 mg) is administered by a slow infusion over at least 2 minutes after 4 hours of oral administration and rosuvastatin tablet (20 mg). Blood samples for analysis of cocktail drugs are drawn at the following time-points: before (0 hours) and 0.25, 0.5, 1, 1.5, 2, 3, 4, 4.25, 4.5, 5, 5.5, 6, 8, 10, 12, 23 and 24 hours following administration. Actual times of cocktail administration as well as blood samplings are registered. The blood samples during the PK investigations for drug analysis are drawn in pre-chilled K2-EDTA vacutainer tubes (no gel or plastic beads) on ice, centrifuged for 10 minutes at 4°C (1800 g), plasma

decanted into Cryovials and frozen within 1 hour at -70°C. Detail dosing, sample collection, sample preparation and analysis is reported previously (10, 11).

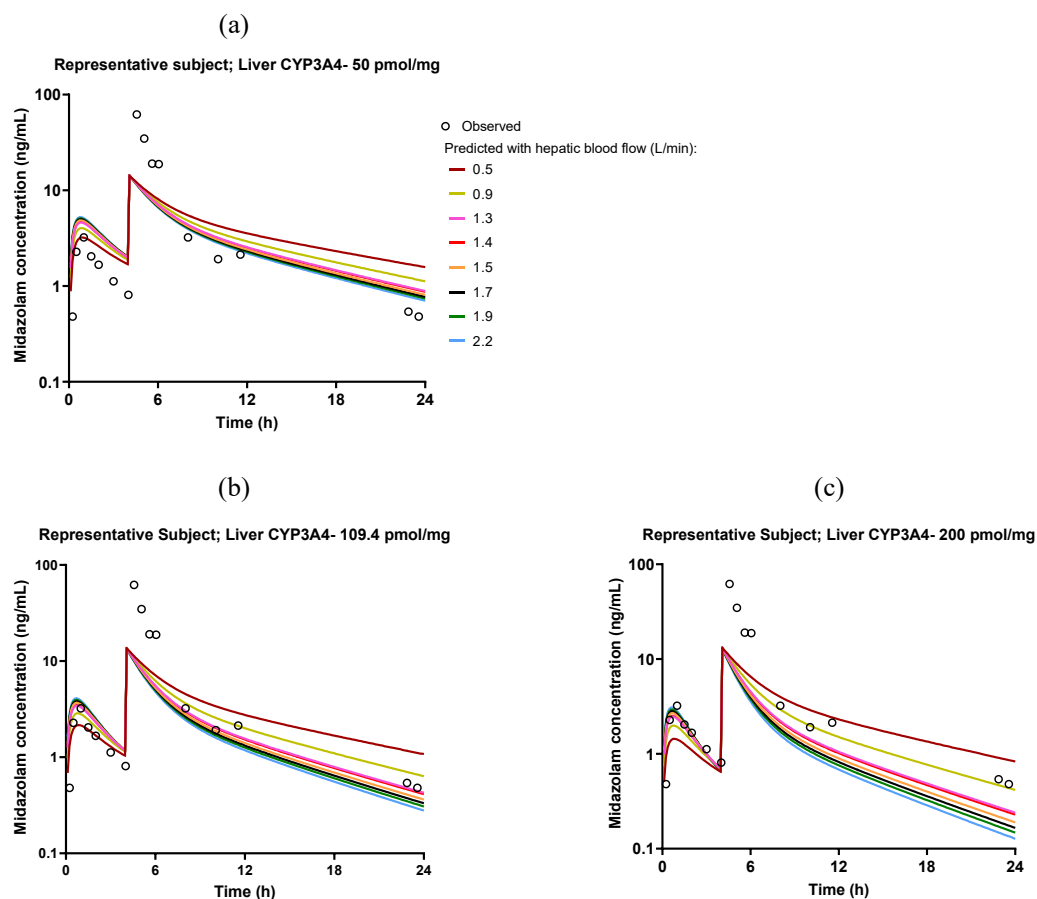


Supplementary Figure 1 Comparison of whole liver weights (a) and lean liver weight (b) between individuals with or without obesity and comparison of whole and lean liver weight from normal weight individuals (non-obese) (c) and individuals with obesity/severe obesity (d) from COCKTAIL trial. The boxplots represent the median (horizontal line within each box), 5th and 95th percentile (box limits), and the minimum and maximum values within the percentile intervals (whiskers). The mean is indicated by a plus sign (+).

**** $p < 0.0001$; ns- not statistically significant ($p > 0.05$).



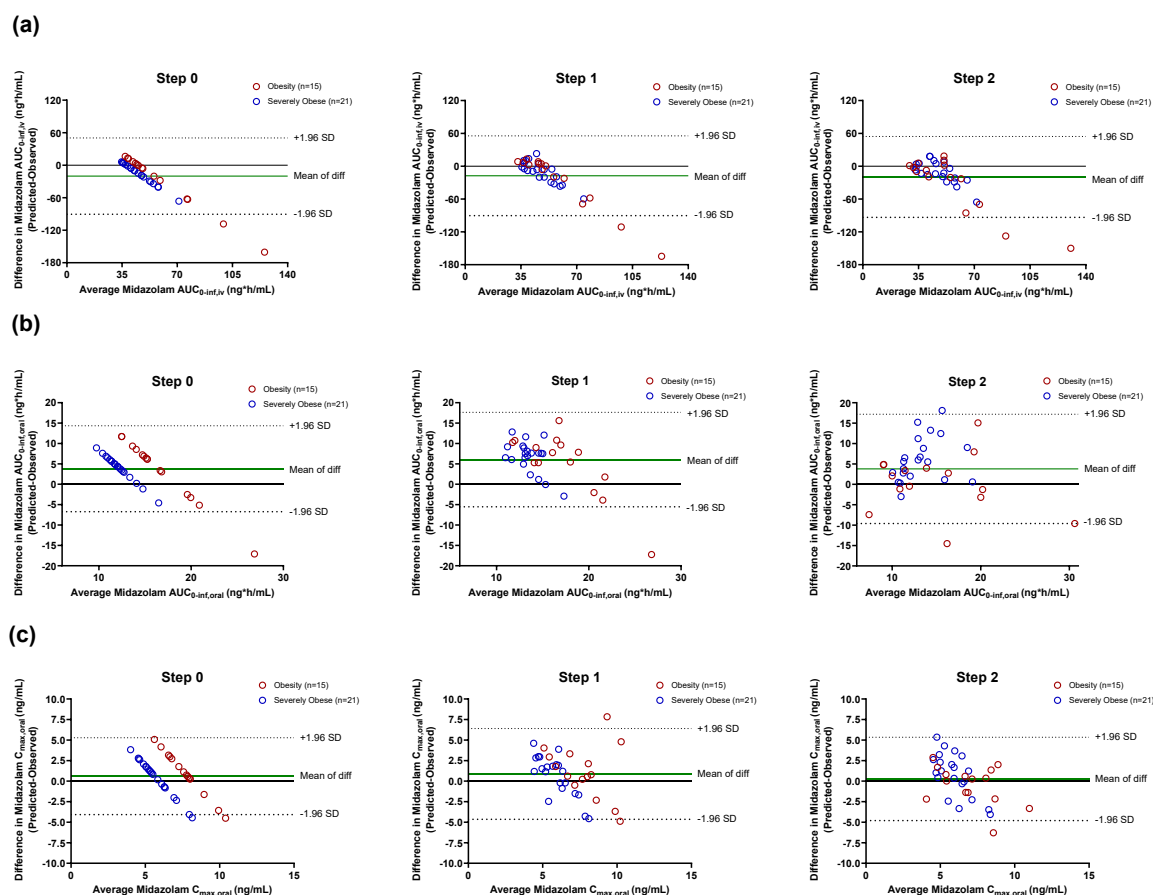
Supplementary Figure 2 Difference between whole liver and lean liver weights in individuals with obesity (a) and severe obesity (b). The boxplots represent the median (horizontal line within each box), 5th and 95th percentile (box limits), and the minimum and maximum values within the percentile intervals (whiskers). The mean is indicated by a plus sign (+). ns- not statistically significant ($p>0.05$).



Supplementary Figure 3: Sensitivity analysis of hepatic blood flow on midazolam PK at varying hepatic CYP3A4 abundances in a representative subject from the COCKTAIL trial. Simulated plasma concentration–time profiles of midazolam are shown under different hepatic blood flow conditions (0.5 to 2.2 L/min) for low (a), intermediate (b) and high (c) hepatic CYP3A4 abundances. The black open circles denote the observed plasma concentrations obtained from COCKTAIL trial for one representative patient.

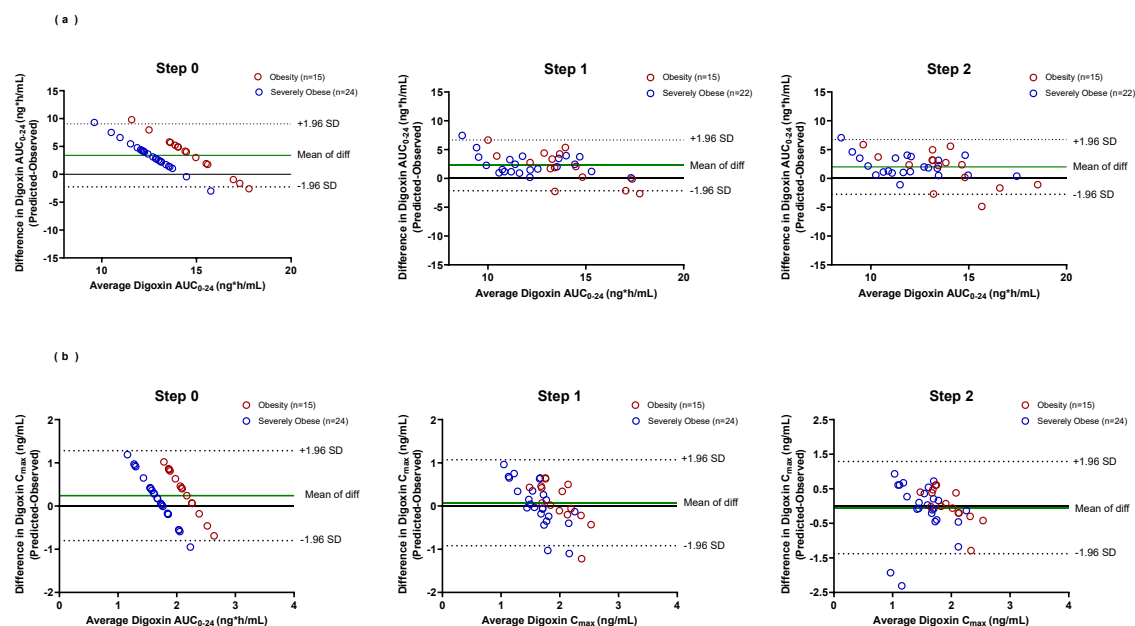
4. Bland-Altman Analysis

Bland–Altman analysis was conducted to assess the agreement between model-predicted and observed PK parameters for midazolam and digoxin in obese and morbidly obese populations. The analysis was performed at each step of the stepwise VT–PBPK modelling approach to evaluate progressive model refinement. For each PK parameter evaluated, the difference between the predicted and observed values was plotted against their mean, following the method described by Bland and Altman (12). The mean difference (bias) and the 95% limits of agreement (LoA), defined as the mean difference $\pm 1.96 \times$ standard deviation (SD) of the differences, were calculated. This allowed quantification of both systematic bias and the degree of dispersion between predicted and observed data.

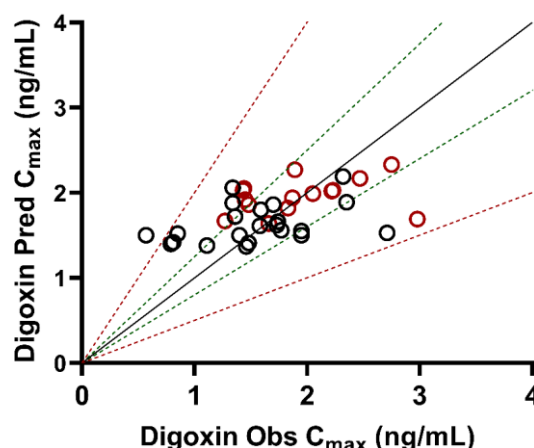


Supplementary Figure 4 Bland-Altman plots comparing predicted and observed midazolam PK parameters across the three model refinement steps in individuals with obesity (red circles, $n = 15$) and severe obesity (blue circles, $n = 21$). (a) Intravenous midazolam AUC0-inf ($AUC_{0-inf,iv}$), (b) oral midazolam AUC0-inf ($AUC_{0-inf,oral}$), and (c) oral midazolam C_{max} ($C_{max,oral}$) are shown for Step 0 (left),

Step 1 (middle), and Step 2 (right). The y-axis represents the difference between predicted and observed values, while the x-axis represents the average of the predicted and observed values. The solid green line indicates the mean difference, and the dotted black lines represent the limits of agreement (mean $\pm$ 1.96 SD). The black solid line represents no difference between predicted and observed values.



Supplementary Figure 5 Bland–Altman plots comparing predicted and observed digoxin PK parameters across the three model refinement steps in individuals with obesity (red circles, $n = 15$) and severe obesity (blue circles, $n = 22$ or 24). (a) Digoxin AUC₀₋₂₄, (b) Digoxin C_{max} are shown for Step 0 (left), Step 1 (middle), and Step 2 (right). The y-axis represents the difference between predicted and observed values, while the x-axis represents the average of the predicted and observed values. The solid green line indicates the mean difference, and the dotted black lines represent the limits of agreement (mean $\pm$ 1.96 SD). The black solid line represents no difference between predicted and observed values.

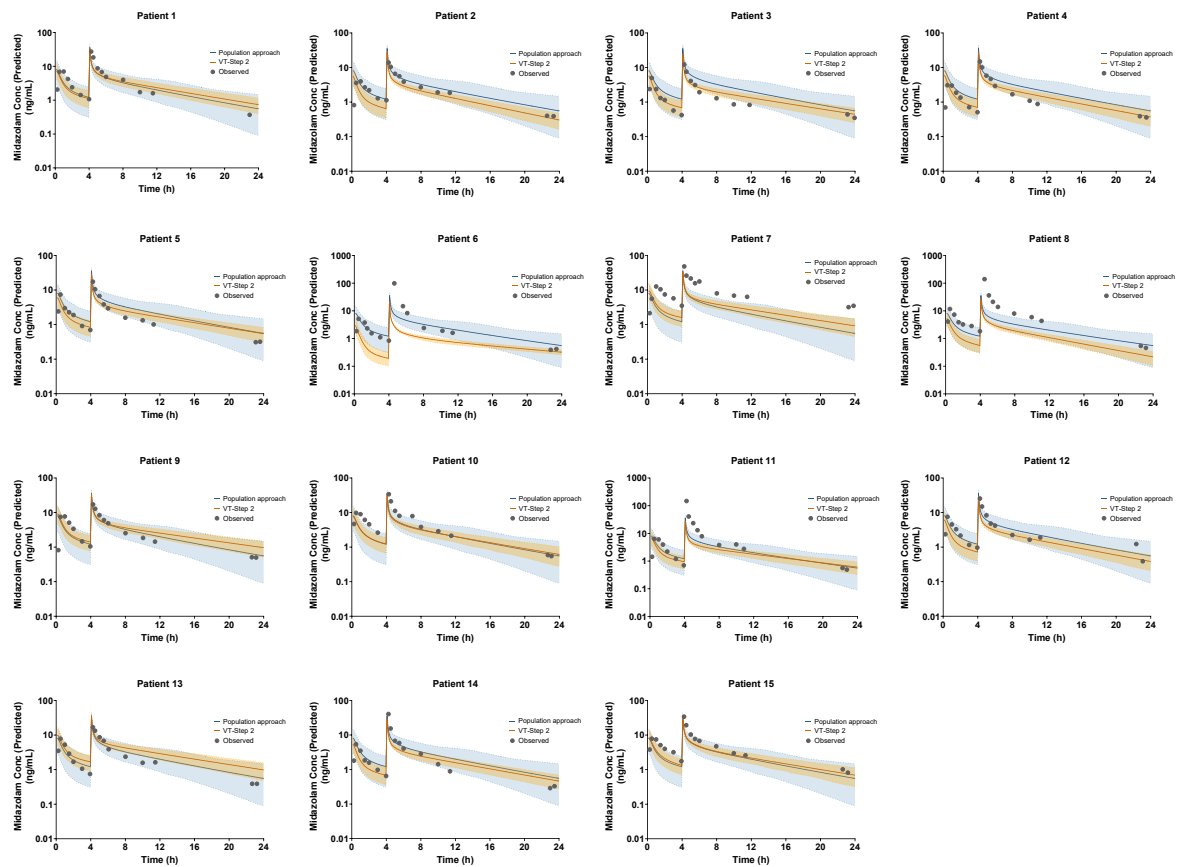


Supplementary Figure 6 Observed versus predicted $C_{\max}$ values for digoxin following Step 2 of the individualization. Red open circle represents an individual with obesity ($n=15$), whereas black represents patients with severe obesity ($n=22$). The solid black line denotes the line of unity, while the green and red dashed lines represent 1.25-fold and 2-fold differences between observed and predicted values, respectively.

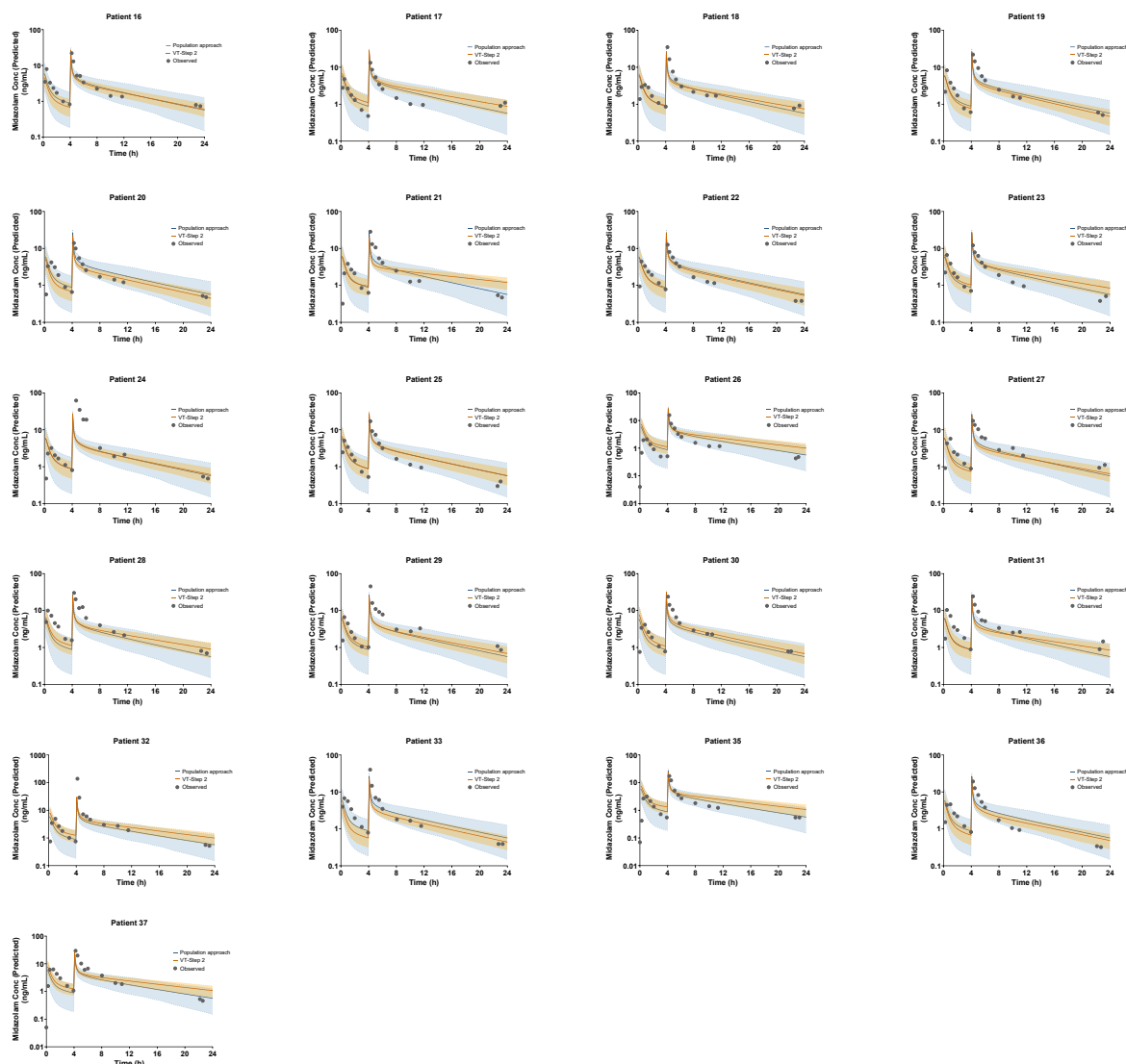
5. Implementation of hepatic blood flow in the PBPK models for obese populations

The obese population files in Simcyp Simulator account for changes in hepatic blood flow by scaling flow rates relative to liver size and body weight, incorporating literature-informed adjustments for these populations. Specifically, hepatic blood flow is implemented as a relative fraction of cardiac output, which itself is adjusted according to obesity status. In contrast, Berton et al. (13) developed a model that explicitly incorporates both absolute hepatic blood flow values and their variation with BMI, based on literature data available. Their analysis demonstrated that absolute liver blood flow increases with BMI up to a certain threshold, but then plateaus or decreases at higher BMI ranges, highlighting a non-linear relationship. This approach differs from the Simcyp model, which is based on relative hepatic blood flow scaling, potentially leading to differences in predicted hepatic clearance in individuals with severe obesity. However, the available clinical data on hepatic blood flow in individuals with severe obesity ($\text{BMI} \geq 40 \text{ kg/m}^2$) remain limited and somewhat inconsistent, posing challenges for accurate model parameterization.

6. Virtual Twin Individual PK Profile Plots

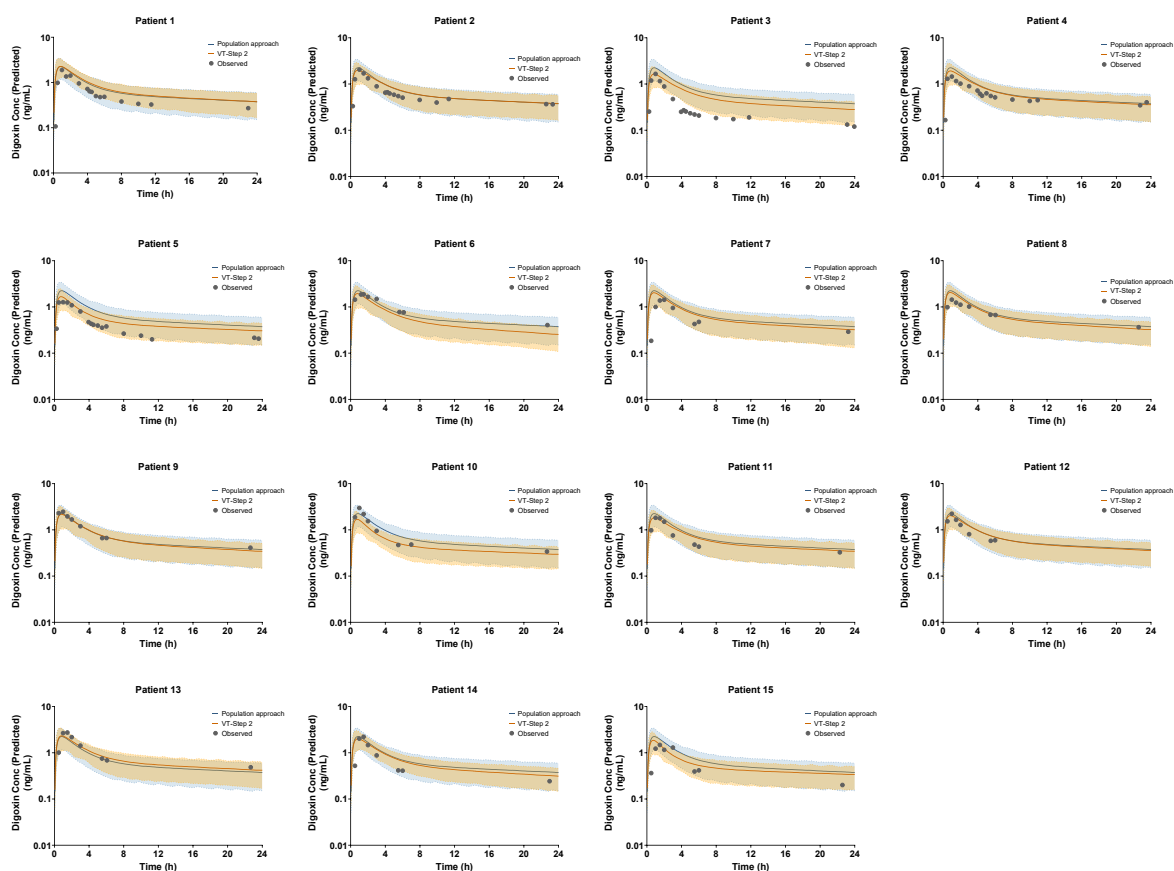


Supplementary Figure 7: Individual plasma concentration-time profiles of midazolam for all the patients with obesity (n=15), displayed on a logarithmic scale. The grey dots denote the observed plasma concentrations obtained from COCKTAIL trial. The solid blue line represents the Step 0 prediction (no individualization), and the solid orange line indicates the Step 2 prediction (model individualized with demographics, proteomic and biochemical data). Blue and orange shaded areas around each line correspond to the 5th and 95th percentile prediction intervals for Step 0 and Step 2, respectively.

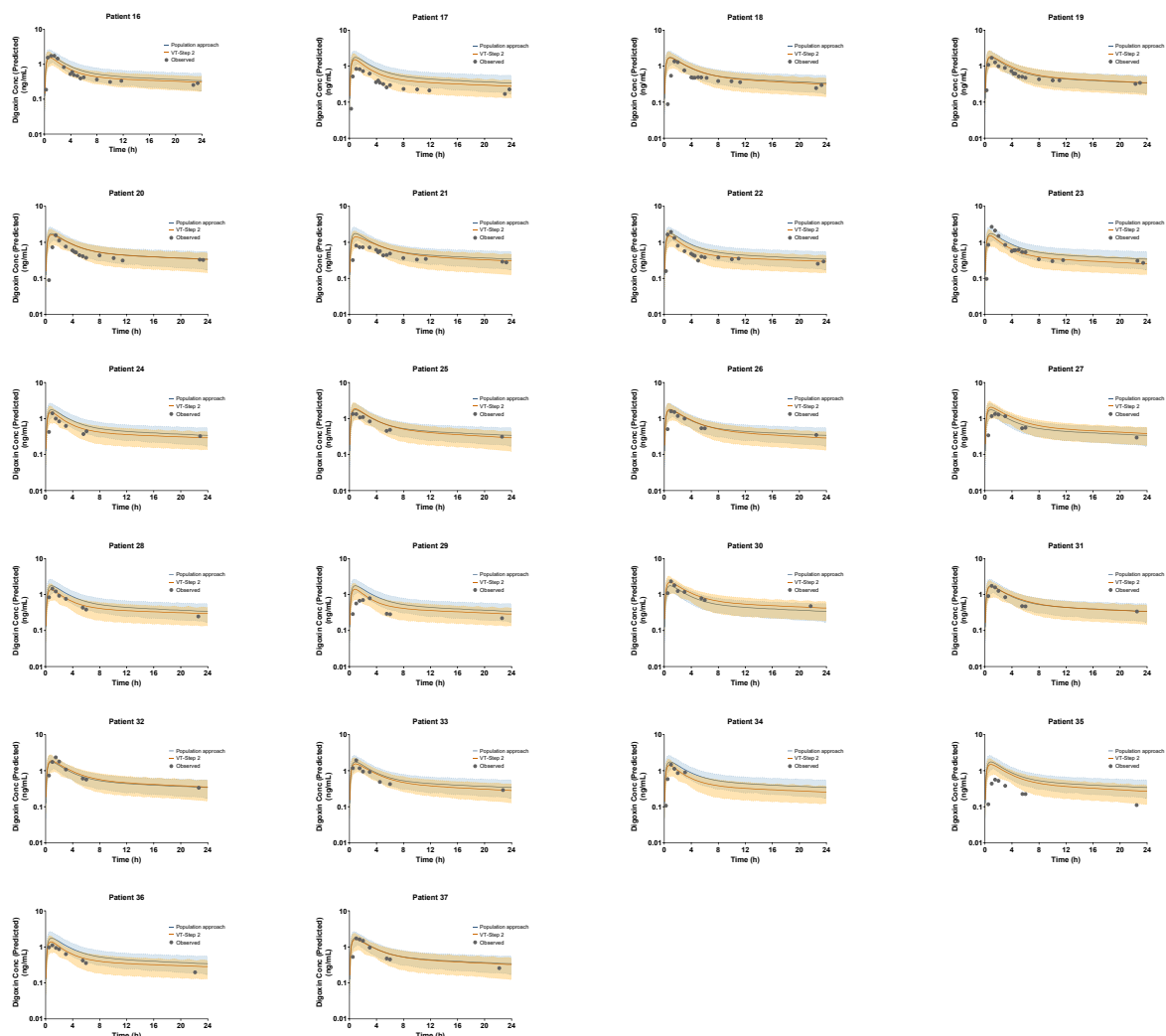


Supplementary Figure 8 Individual plasma concentration-time profiles of midazolam for all the patients with severe obesity (n=21), displayed on a logarithmic scale. The grey dots denote the observed plasma concentrations obtained from COCKTAIL trial. The solid blue line represents the Step 0 prediction (no individualization), and the solid orange line indicates the Step 2 prediction (model individualized with demographics, proteomic and biochemical data). Blue and orange shaded areas around each line correspond to the 5th and 95th percentile prediction intervals for Step 0 and Step 2, respectively.

Note: Patient 34 is missing as no observed PK data for midazolam were available.



Supplementary Figure 9 Individual plasma concentration-time profiles of digoxin for all the patients with obesity (n=15), displayed on a logarithmic scale. The grey dots denote the observed plasma concentrations obtained from COCKTAIL trial. The solid blue line represents the Step 0 prediction (no individualization), and the solid orange line indicates the Step 2 prediction (model individualized with demographics, proteomic and biochemical data). Blue and orange shaded areas around each line correspond to the 5th and 95th percentile prediction intervals for Step 0 and Step 2, respectively.



Supplementary Figure 10 Individual plasma concentration-time profiles of digoxin for all the patients with severe obesity (n=22), displayed on a logarithmic scale. The grey dots denote the observed plasma concentrations obtained from COCKTAIL trial. The solid blue line represents the Step 0 prediction (no individualization), and the solid orange line indicates the Step 2 prediction (model individualized with demographics, proteomic and biochemical data). Blue and orange shaded areas around each line correspond to the 5th and 95th percentile prediction intervals for Step 0 and Step 2, respectively.

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