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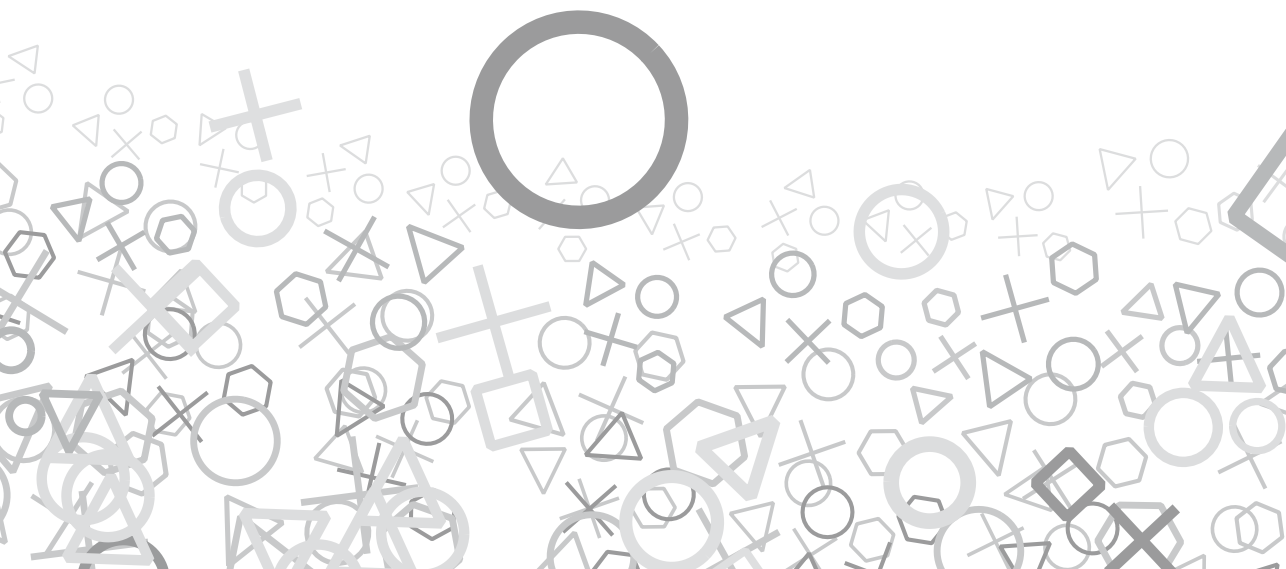
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CHAPTER 7

A semi-physiologically based pharmacokinetic model for midazolam and CYP3A mediated metabolite 1-OH-midazolam in morbidly obese and weight loss surgery patients

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ABSTRACT

This study aimed to describe the pharmacokinetics of midazolam and its CYP3A mediated metabolite 1-OH-midazolam in morbidly obese patients receiving oral and intravenous midazolam before (n=20) and one year after weight loss surgery (n=18), thereby providing insight into the influence of weight loss surgery on CYP3A activity in the gut wall and liver.

In a semi-PBPK model in which different blood flow scenarios were evaluated, intrinsic hepatic clearance of midazolam ($CL_{int\ H}$) was 1.5 times higher compared to morbidly obese patients before surgery ($p < 0.01$). Midazolam gut wall clearance ($CL_{int\ G}$) was slightly lower in patients after surgery ($p > 0.05$), with low values for both groups.

The results of the semi-PBPK model suggest that in patients after weight loss surgery CYP3A hepatic metabolizing capacity seems to recover compared to morbidly obese patients, while CYP3A mediated intrinsic gut wall clearance was low for both populations and showed large inter individual variability.

INTRODUCTION

Weight loss surgery or bariatric surgery is widely and increasingly applied to treat morbid obesity (body mass index $> 40 \text{ kg/m}^2$)^{1, 2, 3}. This type of surgery may profoundly affect drug pharmacokinetics, as the procedure reduces the stomach to a small pouch and, in case of a Roux- and Y-gastric bypass (RYGB), 75-150 cm of the initial part of the small intestines including the duodenum is bypassed^{4, 5}. In addition, patients lose on average 32% of their body weight within 1 year⁶, which may affect clearance and the distribution of drugs as well⁷.

Previously, we showed in a population PK analysis that plasma clearance (CL) of the cytochrome P450 3A (CYP3A) substrate midazolam is 1.7 times increased in patients after a weight loss procedure in comparison to morbidly obese patients, while oral bioavailability (F_{total}) was unaltered⁸. Similar results have been reported before for RYGB patients in comparison with age- gender- and BMI matched control patients⁹. While it is well known that CYP3A resides both in the gut and in the liver, these analyses that use total oral bioavailability (F_{total}) as parameter do not allow for a distinction between the contribution of pre-systemic gut and pre-systemic liver metabolism. More specifically, oral bioavailability (F_{total}) may be deduced to its individual contributors, which are the fraction absorbed (F_a), the fraction escaping gut wall metabolism (F_g) and the fraction escaping first pass hepatic metabolism (F_h). As midazolam is a highly soluble and permeable drug, F_a is assumed to be equal to 1 in morbidly obese patients before and after surgery^{10, 11}. Keeping in mind the reported increase in midazolam systemic plasma clearance after a weight loss surgery^{9, 12}, F_h is expected to decrease after weight loss surgery. So, given the unchanged total bioavailability (F_{total}) identified in these patients¹², it may be hypothesized that the midazolam fraction escaping gut wall (F_g) increases one year after weight loss surgery (Supplementary information 1). In theory, such an increase in F_g upon weight loss surgery may be attributed to the 75-150 cm bypass of the small intestine during an RYGB surgery⁴ potentially causing reduced (intrinsic) CYP3A clearance in the gut.

Knowledge on the exact influence of a weight loss surgery on hepatic and gut wall CYP3A clearance is important because approximately 30% of all clinically used drugs are metabolised via this enzyme¹³. To fully characterise the influence of weight loss surgery on CYP3A mediated drug metabolism in both the gut wall and the liver, a semi physiologically-based pharmacokinetic (semi-PBPK) model taking into account these distinct processes needs to be applied to both midazolam and the CYP3A mediated metabolite 1-OH-midazolam concentrations obtained after oral and intravenous administration in these populations^{14, 15}. Such a semi-PBPK model consists of a compartment representing the gut wall, the portal vein and the liver, and an empirical compartment model for midazolam and 1-OH midazolam, representing the rest of the body. The model is

parameterized on the basis of intrinsic clearance (CL_{int}) for both the gut and the liver, blood flow (Q) and fraction unbound (f_u) in the blood or gut wall. In this model, intrinsic midazolam clearance in the liver or gut wall represents the capacity of the liver or gut wall to metabolize midazolam into 1-OH-midazolam and therefore represents CYP3A activity in these respective organs.

In this study we aimed to describe both midazolam and its CYP3A mediated metabolite 1-OH-midazolam in morbidly obese patients before and one year after weight loss surgery after both oral and intravenous administration using a semi-PBPK model, ultimately to evaluate how the intrinsic CYP3A activity in the gut wall and liver are affected by weight loss surgery and (loss of) body weight. In addition, the results are used to explore to what extent these results may affect other CYP3A substrates used after weight loss surgery.

METHODS

Study design and patients

In this study, data are used from a prospective observational cohort study in 20 morbidly obese patients at the day of laparoscopic weight loss surgery of whom 18 patients were studied again one year later (NTC01519726, EudraCT 2011-003293-93). Study design and characteristics have been described before and are repeated briefly below ¹².

In the study, morbidly obese patients undergoing a laparoscopic gastric bypass or sleeve surgery were eligible for inclusion. Patients were excluded if they used CYP3A inducing or inhibiting medication ¹⁶, used products containing grapefruit, wild grape, banpeiyu, pomegranate, star fruit or black berry within two weeks before the study, were pregnant, gave breastfeeding or suffered from renal insufficiency (eGFR MDRD4 <60 mL/min). Before participation, all patients gave written informed consent. One year after the weight loss procedure 18 of the 20 patients were restudied using the same study design. At both occasions, patients received 7.5 mg oral and 5 mg intravenous midazolam separated by 160 ± 48 minutes. Per patient and occasion, a mean of 22 blood samples were collected to measure both midazolam and 1-OH midazolam concentrations. Plasma concentrations were measured using a method described before ¹⁷. For 1-OH midazolam, the lower limit of quantification was 0.9 ng/mL and intra assay and inter assay coefficients of variation were 6.3% and 4.5%.

The study was approved by the local human research and ethics committee (NL35861.100.11) and was conducted according to the principles of the Declaration of Helsinki (version 22-10-2008) and in accordance with the Medical Research Involving Human Subjects Act (WMO) of the Netherlands.

Population pharmacokinetic modelling

Population pharmacokinetic modelling was performed using NONMEM 7.3¹⁸ ADVAN 6 and (PsN version 3.6.2), Pirana (version 2.9.0) and R (version 3.1.2) to visualize the data. Different structural models were tested to fit the midazolam and 1-OH-midazolam data from morbidly obese patients before and after weight loss surgery.

First, a regular population pharmacokinetic (PK) model was applied with a two-compartment model for 1-OH-midazolam, a three compartment model for midazolam and a transit compartment model for midazolam oral absorption in which oral absorption rate (K_a) was set equal to the transit compartment rate (K_{tr}) (Intermediate model, Figure 1a). This model was based on earlier work on the pharmacokinetics of midazolam not involving the 1-OH-midazolam metabolite¹².

Second, a semi physiologically based pharmacokinetic (semi-PBPK) model was applied to describe the data (Semi-PBPK model, Figure 1b). The structural semi-PBPK model was adopted from Yang *et al.* (2003) and Frechen *et al.* (2013) and consisted of a compartment representing the gut wall, the portal vein and the liver, and an empirical compartment model for midazolam and 1-OH midazolam, representing the rest of the body^{14,15}. In order to reduce runtimes, midazolam and 1-OH-midazolam were assumed to reach an instant equilibrium in the gut wall, portal vein and liver compartment which resulted in a simplified semi-PBPK model (Supplemental information 2). For midazolam, a three compartment model was used and for midazolam oral absorption a transit compartment model in which the oral absorption rate was equalized to the transit compartment rate (K_{tr})¹⁹ was used. For 1-OH-midazolam, a two compartment model was applied.

In the semi-PBPK model, hepatic (E_H) and the gut wall extraction (E_G) of midazolam were defined as the input for the liver and gut wall compartment of the 1-OH-midazolam model, respectively. Hepatic extraction of midazolam (E_H) and 1-OH-midazolam ($E_{H, 1-OH}$) was defined by the well-stirred model:

$$E_H = \frac{CL_{int,H} \times fu}{Q_{H,B} + (CL_{int,H} \times fu_B)} \quad (\text{Eq. 1})$$

where $CL_{int,H}$ is the intrinsic hepatic clearance based on unbound blood concentrations, fu_B is the unbound concentration in blood and $Q_{H,B}$ is the hepatic blood flow.

The fraction escaping hepatic metabolism (F_H) was defined as:

$$F_H = 1 - E_H \quad (\text{Eq. 2})$$

For gut wall midazolam metabolism into 1-OH-midazolam (E_G) the Q_{Gut} model was used²⁰:

A

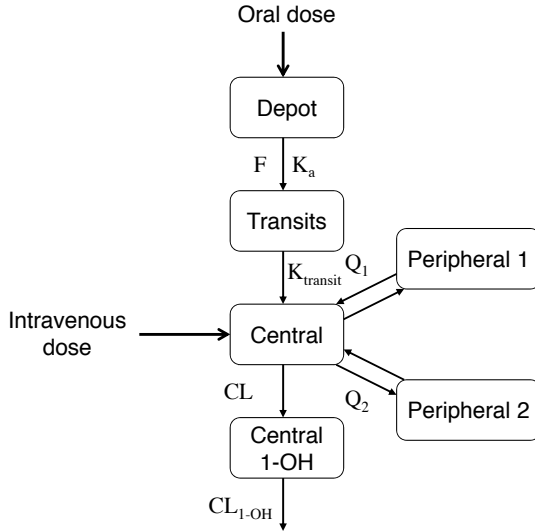
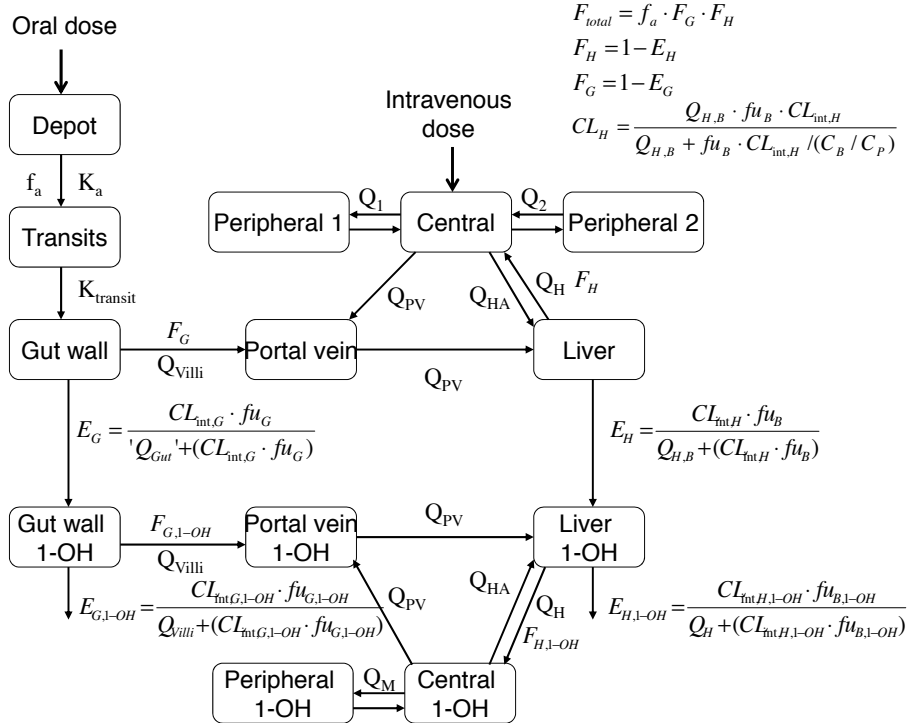


Figure 1 Schematic representation of the intermediate population pharmacokinetic model (a) and semi-PBPK model (b) for midazolam and its 1-OH-midazolam metabolite (1-OH). B= blood; CL_{int} = intrinsic clearance; E= extraction ratio; G= gut wall; F= bioavailability; f_s = fraction absorbed into the gut wall; f_u = fraction unbound; H= hepatic; HA= hepatic artery; K_a = oral absorption rate; $K_{transits}$ = transit compartment rate; Q is blood flow (Q_{villi} , Q_{PV} , Q_{HA} , Q_H) or intercompartmental clearance (Q_1 and Q_2); PV= portal vein.

B



$$E_G = \frac{CL_{int,G} \times fu_G}{Q_{Gut} + (CL_{int,G} \times fu_G)} \quad (\text{Eq. 3})$$

where $CL_{int,G}$ is the intrinsic gut wall clearance based on unbound blood concentrations, fu_G is the unbound drug concentration in the gut wall and Q_{Gut} is defined by ²⁰:

$$Q_{Gut} = \frac{Q_{villi} \times CL_{perm}}{Q_{villi} + CL_{perm}} \quad (\text{Eq. 4})$$

where, Q_{villi} is the villous blood flow and CL_{perm} is a term defining the permeability of the drug through the enterocytes in the gut wall. The fraction escaping gut wall metabolism was defined as:

$$F_G = 1 - E_G \quad (\text{Eq. 5})$$

Gut wall extraction of 1-OH-midazolam ($E_{G, 1-OH}$) was defined by:

$$E_{G, 1-OH} = \frac{CL_{int,G, 1oh=OH} \times fu_{G, 1OH}}{Q_{villi} \times (CL_{int,G, 1OH} + fu_{G, 1OH})} \quad (\text{Eq. 6})$$

Systemic plasma clearance (CL_H) was derived from the hepatic midazolam intrinsic clearance and hepatic blood flow using ²¹:

$$CL_H = \frac{Q_{H,B} \times fu_B \times CL_{int,H}}{Q_{H,B} + fu_B \times CL_{int,H} / (C_B / C_P)} \quad (\text{Eq. 7})$$

In which C_B/C_P is the blood to plasma ratio.

Values used for the drug parameters are listed in Table 1. The fraction of midazolam absorbed (F_a) was fixed to 1 and it was assumed that no protein binding occurred in the gut wall (Table 1). As midazolam is an intermediate extraction ratio drug ($E_H = \sim 0.4$ ^{22, 23, 24}), for the hepatic blood flow (Q_H) three different scenarios were explored including Q_H based on allometric scaling (scenario 1) ²⁵, Q_H based on a model for cardiac output in obese and morbidly obese patients (scenario 2) ^{26, 27} and a Q_H that was the same before and after weight loss surgery (scenario 3), see Table 1.

Discrimination between different structural models was made by comparison of the objective function value (OFV, i.e. -2 log likelihood [-2LL]). A p-value below 0.05, representing a decrease of 3.84 in the OFV between nested models for one degree of freedom, was considered statistically significant. In addition, goodness-of-fit plots (observed versus individual-predicted concentrations, observed versus population-

predicted concentrations, conditional weighted residuals versus time and conditional weighted residuals versus population-predicted concentrations plots) of midazolam and 1-OH-midazolam were used for diagnostic purposes. Furthermore, the confidence interval of the parameter estimates, the correlation matrix and visual improvement of the individual plots were used to evaluate the models. The internal validity of the population pharmacokinetic model was assessed by the bootstrap re-sampling method using 500 replicates.

For the statistical model, the individual parameter estimate (empirical bayes estimate or post hoc value) of the *i*th individual was modelled according to:

$$\theta_i = \theta_{mean} \times e^{\eta_i} \quad (\text{Eq. 8})$$

Table 1 Values used for drug parameters and for three hepatic blood flow scenarios used in the semi-PBPK model

Parameter (unit) reference	Scenario 1 Allometric scaling of the hepatic blood flow ²⁵	Scenario 2 Hepatic blood flow as a fraction of cardiac output ^{26, 27}	Scenario 3 One hepatic blood flow for all individuals
<i>Midazolam</i>			
<i>f_a</i>		1 ²³	
B:P		0.66 ^{23, 42}	
<i>f_{uG}</i>		1	
<i>f_{uB}</i>		0.0303 ⁴²	
CL _{perm} (L/min)		0.177 ²⁰	
<i>1-OH-midazolam</i>			
B:P		1	
<i>f_{uG, 1-OH}</i>		1 ²⁰	
<i>f_{uB, 1-OH}</i>		0.106 ⁴³	
CL _{perm}		1	
<i>Blood flows</i>			
Cardiac output (L/min) -		(9119– EXP(9.164 + -2.9*10 ⁻² * TBW + 3.91*10 ⁻⁴ * TBW ² + -1.91*10 ⁻⁶ * TBW ³)/1000	7
<i>Q_{hepatic}</i> (L/min)	3.75 * TBW ^{0.75}	0.25 * CO ²⁷	0.25 * CO ²⁷
<i>Q_{hepatic artery}</i>	0.25 * <i>Q_{hepatic}</i> ²⁷	0.25 * <i>Q_{hepatic}</i> ²⁷	0.25 * <i>Q_{hepatic}</i> ²⁷
<i>Q_{portal vein}</i>	0.75 * <i>Q_{hepatic}</i> ²⁷	0.75 * <i>Q_{hepatic}</i> ²⁷	0.75 * <i>Q_{hepatic}</i> ²⁷
<i>Q_{small intestine}</i>	0.4 * <i>Q_{hepatic}</i> ²⁷	0.1 * CO ^{20, 27}	0.4 * <i>Q_{hepatic}</i> ²⁷
<i>Q_{mucosal}</i>	0.80 * <i>Q_{small intestine}</i> ²⁰	0.80 * <i>Q_{small intestine}</i> ²⁰	0.80 * <i>Q_{small intestine}</i> ²⁰
<i>Q_{villi}</i>	0.60 * <i>Q_{mucosal}</i> ²⁰	0.60 * <i>Q_{mucosal}</i> ²⁰	0.60 * <i>Q_{mucosal}</i> ²⁰

B:P = blood to plasma ratio; CO= cardiac output; *f_a* = fraction absorbed in the gut wall; *f_{ub}* = fraction unbound in blood; *f_{uG}* = fraction unbound in gut wall; CL_{perm} = parameter representing the permeability through the enterocyte; *Q*= blood flow; TBW= total body weight.

where θ_{mean} is the population mean, and η_i is a random variable for the i th individual with a mean of zero and variance of ω^2 , assuming log-normal distribution in the population. For residual variability, resulting from assay errors, model misspecifications and other unexplained sources, a proportional error model was used. The j th observed midazolam concentration of the i th individual (Y_{ij}) is described by:

$$Y_{ij} = C_{\text{pred},ij} \times (1 + \varepsilon_{ij}) \quad (\text{Eq. 9})$$

where $C_{\text{pred},ij}$ is the individual predicted midazolam concentration of the i th individual at the j th time, and ε_{ij} is a random variable with a mean of zero and variance of σ^2 .

Data below the limit of quantification of the bio-analysis assay were provided by the lab and included in the data set. Data below the limit of detection, defined as 30% of the lower limit of quantification, were deleted from the data set (5.7% for midazolam and 8.9% for 1-OH-midazolam) ²⁸.

Based on our earlier pharmacokinetic analysis for midazolam, body weight on midazolam central and peripheral volume of distribution for morbidly obese patients and a separate parameter estimate for midazolam oral absorption rate and inter-compartmental clearance in morbidly obese and weight loss patients were included in the model ⁸. After inclusion of these midazolam covariates, the influence of weight loss surgery was evaluated for midazolam gut wall and hepatic intrinsic clearance (CL_{int}). The binary covariate before/after weight loss surgery was plotted independently against the individual *post hoc* values and eta estimates of midazolam intrinsic clearance estimates to visualize potential relations. The covariate 'before/after weight loss surgery' was tested by means of a separate parameter or using the following equation:

$$P_i = P_p \times Z^{\text{COV}} \quad (\text{Eq. 10})$$

where P_i and P_p represent individual and population parameter estimate, Z the estimated factor of increase or decrease for the patients subgroup with COV equalling one.

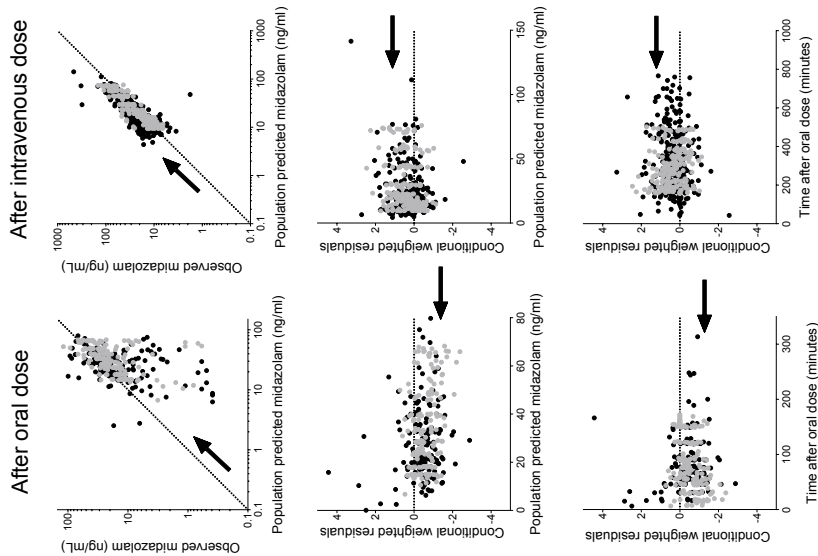
Potential covariates were separately entered into the model and statistically tested ($p < 0.05$) by use of the OFV and, if applicable, the 95% confidence interval of the additional parameter. In addition, if applicable, it was evaluated whether the inter-individual variability (eta) in the parameter concerned decreased upon inclusion of the covariate on the parameter and whether the trend in the eta versus covariate plot had resolved.

SimCYP simulations

The influence of weight loss surgery on mean systemic plasma clearance values of other CYP3A substrates was evaluated using the morbidly obese population in the SimCYP software and manipulation of the value for CYP3A hepatic abundance ^{29, 30}. For each CYP3A mediated drug, 10 trials of 10 individuals were simulated.

a

Midazolam



1-OH-midazolam

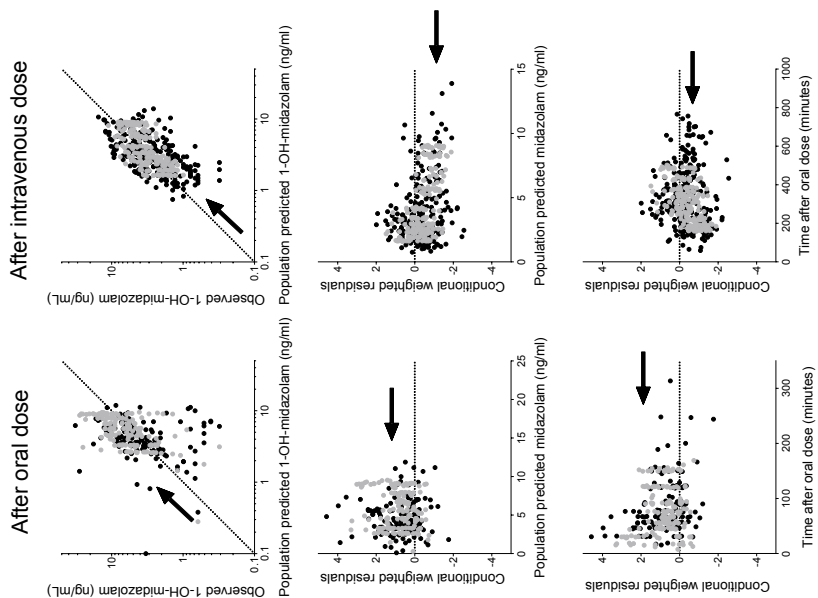
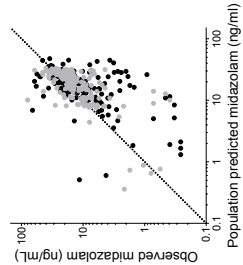


Figure 2 (a) Goodness-of-fit plots for midazolam (left) and 1-OH-midazolam (right) plasma concentrations for the population PK model (intermediate model, Figure 1a) for morbidly obese (black dots) and weight loss patients (grey dots), including population predicted versus observed plots (upper row), population predicted concentrations versus conditional weighted residuals (middle row) and time after oral dose versus conditional weighted residuals (lower row). The arrows indicate the direction of model misspecification. (b) Goodness-of-fit plots for midazolam (left) and 1-OH-midazolam (right) blood concentrations for the final concentrations for the final semi-PBPK model (Figure 1b) for morbidly obese (black dots) and weight loss

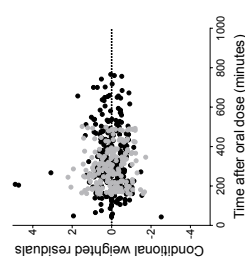
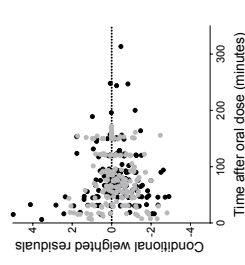
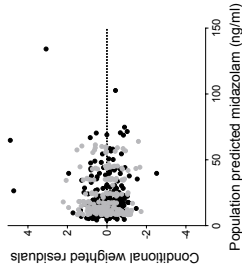
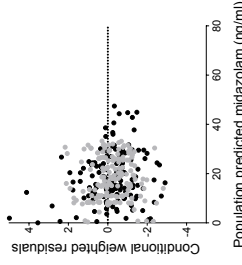
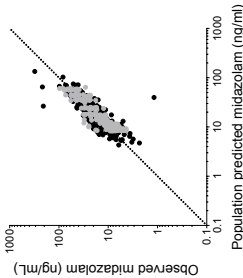
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Midazolam

After oral dose

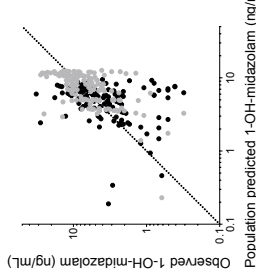


After intravenous dose

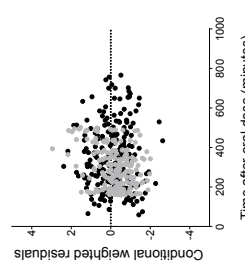
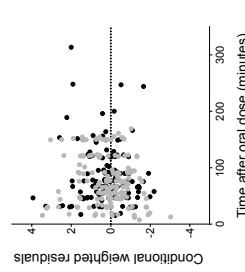
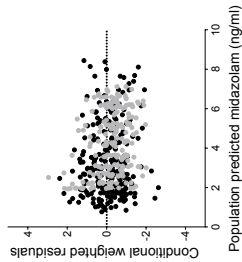
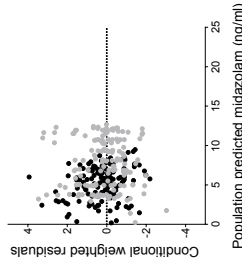
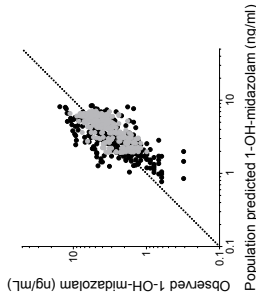


1-OH-midazolam

After oral dose



After intravenous dose



patients (grey dots), including population predicted versus observed plots (upper row), population predicted versus conditional weighted residuals (middle row) and time after oral dose versus conditional weighted residuals (lower row).

RESULTS

Figure 2a shows the goodness-of-fit plots of the midazolam and 1-OH-midazolam plasma concentrations of both morbidly obese and weight loss patients on the basis of the regular population PK model as shown in Figure 1a (Intermediate model). These goodness-of-fit plots show that after the oral dose, midazolam concentrations were over-predicted, while midazolam concentrations after the intravenous dose were under-predicted (Figure 2a). In contrast, 1-OH-midazolam concentrations after oral dose were under-predicted by the model, while 1-OH-midazolam concentrations after intravenous dose were over-predicted. The obvious misspecification of midazolam and its 1-OH-midazolam metabolite concentrations indicate the presence of substantial presystemic 1-OH-midazolam formation after oral administration and therefore, as a second step, a

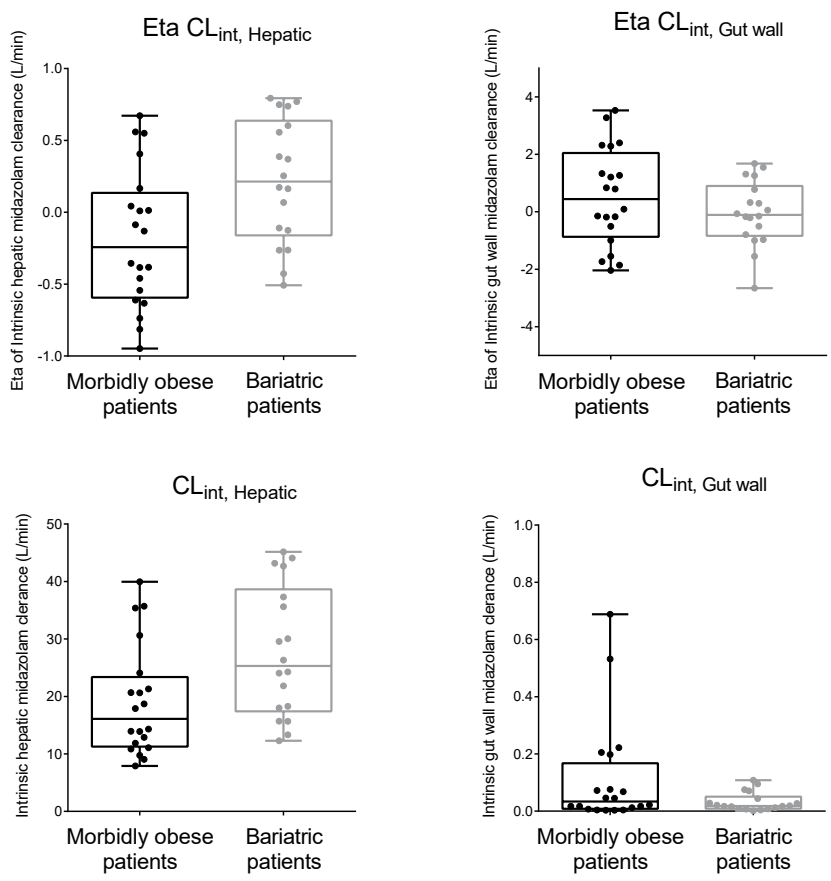


Figure 3 Box and whisker plots of eta and post hoc parameter estimates before addition of covariate effects for intrinsic hepatic (CL_{int,Hr}, left panels, shrinkage of 1%) and gut wall (CL_{int,Gr}, right panels, shrinkage of 21%) midazolam clearance in morbidly obese patients before (black) and after weight loss surgery (grey).

semi-PBPK model including both pre-systemic midazolam metabolism at gut wall and hepatic level was applied (Figure 1b and Supplementary information 3). The goodness-of-fit plots of the semi-PBPK model showed a substantial improvement in the prediction of midazolam and 1-OH-midazolam concentrations after both oral and intravenous dose (Figure 2b).

Upon these findings, the semi-PBPK model was further explored for covariates, taking into account the different Q_H scenarios for obesity (see Methods and Table 2). The influence of weight loss surgery on midazolam intrinsic gut wall ($CL_{int, G}$) and hepatic clearance ($CL_{int, H}$) was evaluated by visual inspection of eta versus covariate plots. Figure 3 shows a trend of higher $CL_{int, H}$ and slightly lower $CL_{int, G}$ in weight loss patients in comparison to morbidly obese patients (Figure 3, upper panels). A separate parameter estimate for $CL_{int, H}$ for morbidly obese and weight loss patients showed a 1.5 times higher

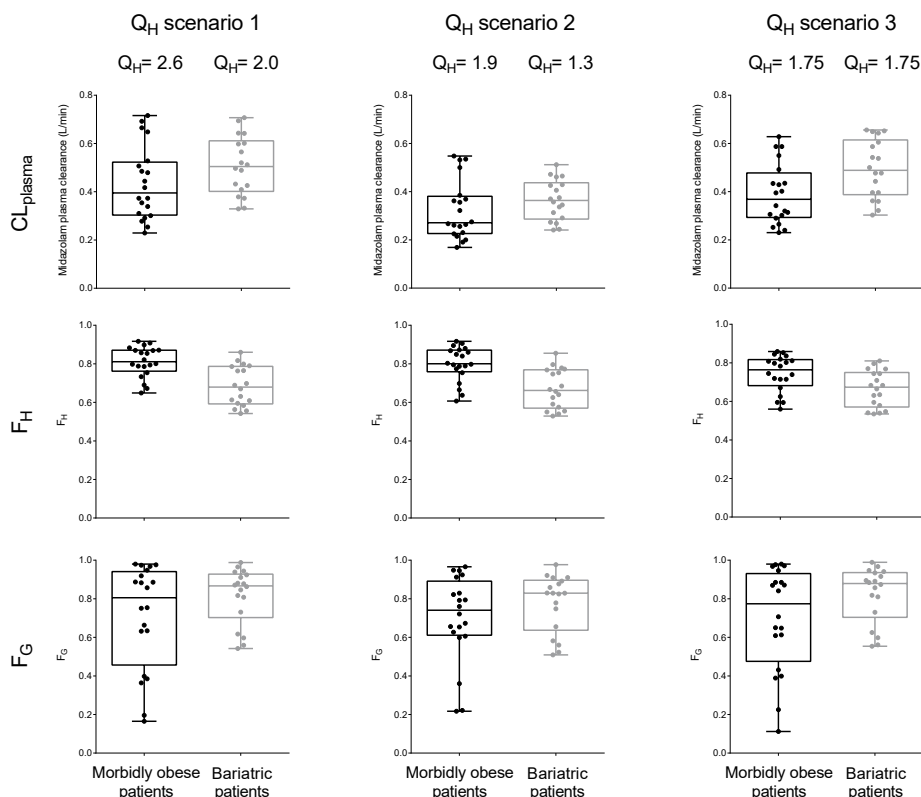


Figure 4 Box and whisker plots of calculated midazolam plasma clearance (equation 7, upper panels), F_H (equation 1 and 2, middle panels) and F_G (equation 3 and 5, lower panels) for morbidly obese (black) and weight loss patients (grey) for three different blood flow scenarios (Q_H in L/min, Table 1). Per scenario the value for hepatic blood flow (Q_H) is shown for the median morbidly obese (144 kg) and median weight loss patient (98 kg) of the studied populations.

Table 2 Blood parameter estimates of the final semi-PBPK model including covariates for scenario 1.

Parameter	Parameter definition	Value (RSE%)	Bootstrap Value (SE)
<i>Midazolam</i>			
$CL_{int,H}$ morbidly obese (L/min)	Intrinsic hepatic clearance morbidly obese	16.8 (14%)	16.9 (2.4)
$CL_{int,H}$ weight loss patients (L/min)	Intrinsic hepatic clearance weight loss patients	25.5 (15%)	25.4 (4.2)
$CL_{int,G}$ (L/min)	Intrinsic gut wall clearance	0.0199 (35%)	0.0207 (0.007)
K_a morbidly obese = K_{tr} (min^{-1})	Oral absorption rate	0.126 (10%)	0.126 (0.01)
K_a weight loss patients = K_{tr} (min^{-1})	Oral absorption rate	0.242 (9%)	0.241 (0.02)
$V_{central}$ weight loss patients (L)	Central midazolam volume of distribution	66.9 (13%)	68.1 (8.7)
$V_{central}$ morbidly obese = $V_{central, 144 \text{ kg}} * (1 + X(TBW - 144))$			
$V_{central, 144 \text{ kg}}$ (L) = $V_{central}$ weight loss patients	Central midazolam volume of distribution for a 144 kg individual	66.9 (13%)	68.1 (8.7)
X	Covariate effect of TBW on $V_{central}$	0.0435 FIX	0.0435 FIX
$V_{peri 1}$ weight loss patients (L)	First peripheral volume of distribution	31.0 (19%)	32.0 (6.5)
$V_{peri 1}$ morbidly obese = $V_{peri 1, 144 \text{ kg}} * (TBW/144)^Y$			
$V_{peri 1, 144 \text{ kg}}$ (L) = $V_{peri 1}$ weight loss patients	First peripheral volume of distribution	31.0 (19%)	32.0 (6.5)
Y	Exponent of covariate function	3.93 FIX	3.93 FIX
$V_{peri 2}$ (L) = $V_{peri 1} * Z$	Second peripheral volume of distribution		
Z		10.8 (13%)	11.1 (1.7)
Q_1 (L/min)	First inter-compartmental clearance	1.41 (15%)	1.35 (0.2)
Q_2 (L/min) = $Q_1 * A$	Second inter-compartmental clearance		
A		3.22 FIX	3.22 FIX
<i>1-OH-Midazolam</i>			
$V_{central, 1-OH}$ (L)	Central volume of distribution	41.7 (11%)	41.9 (4.7)
$V_{peri, 1-OH}$ (L)	Peripheral volume of distribution	16.4 (25%)	17.4 (4.4)
Q_{1-OH} (L/min)	Inter compartmental clearance	0.652 (23%)	0.65 (0.15)
$CL_{int,H,1-OH}$ (L/min)	Intrinsic hepatic clearance	27.4 (9%)	27.2 (2.6)
$CL_{int,G,1-OH}$ (L/min)	Intrinsic gut wall clearance	11.9 (180%)	$4.7 * 10^{22}$ ($7.3 * 10^{23}$)
<i>Inter individual variability</i>			
K_a (%)	Oral absorption rate	44 (20%)	43 (19%)
$V_{central}$ (%)	Central volume of distribution	63 (49%)	61 (38%)
$V_{peripheral 1}$ (%)	First peripheral volume of distribution	113 (24%)	115 (49%)
$CL_{int,H}$ (%)	Intrinsic hepatic clearance	48 (21%)	47 (20%)
$CL_{int,G}$ (%)	Intrinsic gut wall clearance	493 (35%)	582 (168%)
<i>Residual variability</i>			
Morbidly obese patients (%)		32.6 (18%)	32.2 (13%)
Weight loss patients (%)		23.7 (8%)	23.6 (7%)

TBW= total body weight (kg)

intrinsic hepatic clearance in weight loss patients ($-7 \Delta\text{OFV}$, $p < 0.01$ for all Q_H scenarios) and a small decrease in inter individual variability (53% (relative standard error, RSE, of 19%) versus 48% (19%) for scenario 1). A separate parameter estimate for midazolam gut wall intrinsic clearance ($CL_{\text{int,G}}$) did not significantly improve the model ($-2 \Delta\text{OFV}$, $p > 0.05$ for all Q_H scenarios). The two highest values for $CL_{\text{int,G}}$ (see Figure 3, lower row, right plot) are two morbidly obese individuals for which the duration in between oral and intravenous midazolam dose was only 43 and 50 minutes as compared to a mean of 171 ± 57 minutes for the other 18 morbidly obese patients. In addition, it seems that also these two individuals substantially contribute to the uncertainty of the parameter for intrinsic gut wall clearance of 1-OH-midazolam ($CL_{\text{int,G 1-OH}}$). Upon exclusion of these two individuals $CL_{\text{int,G 1-OH}}$ this parameters changes from 11.9 (180%) L/min to 6.7 (40%) L/min. However, exclusion of the two individuals resulted in the same final covariate model, and therefore for the final model all individuals were kept in the data.

Overall, the different Q_H scenarios (see Methods) resulted in slightly different hepatic intrinsic clearance estimates (16.9 (13%), 17.1 (13%) and 12.6 (16%) L/min for morbidly obese patients and, 25.6 (16%), 25.7 (16%) and 18.9 (21%) L/min for weight loss patients for scenario 1, 2 and 3 respectively), while the observed covariate trend between morbidly obese patients and weight loss patients was identical for the different scenarios. Other model parameters and the goodness-of-fit plots were very similar across the sce-

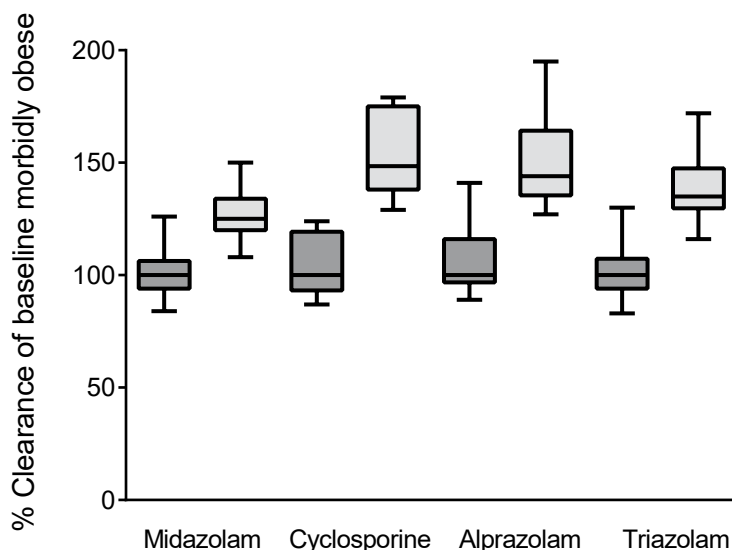


Figure 5 Box and whisker plots of simulated baseline systemic plasma clearances for the SimCYP morbidly obese patient population (dark grey boxes) and percentage change from baseline when hepatic CYP3a abundance is increased by 1.5 times for the morbidly obese population in the SimCYP simulator (light grey boxes) for four CYP3A substrates.

narios. These Q_H scenarios were tested to evaluate the influence of Q_H in the parameters of the model because there is no consensus yet on the exact changes in Q_H upon morbid obesity and subsequent weight loss surgery. While there is no persuasive argument for choosing one Q_H scenario above another, the final parameter estimates and bootstrap results (98% successful) of scenario 1 are presented in Table 2 and goodness-of-fit plots of the this final model are shown in Figure 2b.

The different scenarios for hepatic blood flow slightly influenced the calculated values for midazolam plasma clearance, F_H and F_G , even though the differences between the morbidly obese patients and weight loss patients per scenario remained quite similar (Figure 4). In general for weight loss patients, higher midazolam plasma clearance (upper row, a median increase of 1.28, 1.34 and 1.33, for scenario 1, 2 and 3, respectively) and lower F_H (middle row, median decrease of 0.84, 0.83 and 0.88, respectively) was observed. F_G seems to be close to one for weight loss patients, while the morbidly obese patient group exhibits large inter individual variability (lower row).

Finally, the influence of weight loss surgery on hepatic CYP3A activity was further explored using the SimCYP simulator²⁹. Based on the findings for hepatic intrinsic midazolam clearance of the semi-PBPK model, CYP3A abundances in the liver of the 'morbidly obese population' was 1.5 times increased and plasma clearance values for midazolam, cyclosporine, alprazolam and triazolam were simulated. Figure 5 shows that this increase in CYP3A abundance resulted in a 1.22 increase of midazolam plasma clearance and a median 1.41, 1.37 and 1.30 increase of plasma clearance for CYP3A substrates cyclosporine, alprazolam and triazolam, respectively.

DISCUSSION

In this study we aimed to characterize the pharmacokinetics of both midazolam and its primary CYP3A mediated metabolite 1-OH-midazolam after oral and intravenous administration in morbidly obese patients before and one after weight loss surgery, ultimately to evaluate how intrinsic CYP3A activity in the gut wall and liver are affected by weight loss surgery. We found that midazolam and 1-OH-midazolam concentrations could not be described by a regular compartmental model (Figure 1a, Figure 2a) because of presystemic formation of the CYP3A mediated metabolite 1-OH midazolam for which a semi-PBPK model (Figure 1b, Figure 2b) was needed. Using this model, it was found that midazolam intrinsic hepatic clearance ($CL_{int,H}$) was 1.5 times higher in patients after weight loss surgery, independent of the Q_H scenarios used. In addition, intrinsic midazolam gut wall clearance ($CL_{int,G}$) showed a trend towards lower values in patients after surgery ($p>0.05$).

Intrinsic hepatic midazolam clearance ($CL_{int,H}$) represents the capacity of the liver to metabolize midazolam into 1-OH-midazolam and therefore represents hepatic CYP3A activity. The estimated $CL_{int,H}$ for weight loss patients (25.6, 25.7 and 18.9 L/min for scenario 1, 2 and 3, respectively) was in close agreement with the value reported for healthy volunteers using a very similar semi-PBPK model (27.3 (24.3-30.7) L/min)¹⁵. However, for morbidly obese patients, $CL_{int,H}$ was lower (16.9, 17.1 and 12.6 L/min for scenario 1, 2 and 3, respectively), indicating that hepatic CYP3A activity is reduced in morbidly obese patients in comparison to healthy volunteers but normalizes one year after weight loss surgery. While this recovery of CYP3A activity in the liver upon weight loss surgery has not been reported before, the reduced hepatic CL_{int} due to morbid obesity is supported by in vitro studies showing that human livers samples with steatosis show reduced CYP3A activity in comparison to liver samples without steatosis^{31, 32}. Comparing the observed 1.5 times increase in $CL_{int,H}$ after a weight loss surgery with reported values for midazolam plasma clearance from earlier reports on weight loss surgery patients, it appears that this value closely resembles the previously reported 1.7 times increase in midazolam plasma clearance (CL_{plasma})^{8, 9}. However, when calculating midazolam plasma clearance on the basis of midazolam $CL_{int,H}$ using equation 7, we only find 1.28 increase (scenario 1 and Figure 4). Also, when increasing hepatic CYP3A abundance by 1.5 times in the morbidly obese population of the SimCYP simulator, midazolam plasma clearance only increased 1.22 times. This implies that the increase in midazolam plasma clearance after a weight loss surgery cannot be solely attributed to a normalization or recovery of hepatic CYP3A activity. Therefore, it may be suggested that another non-CYP3A related process may be involved. This other process may be hepatic blood flow (Q_H) or perfusion³³. In the case of patients after weight loss surgery, potentially an improvement in hepatic microcirculation function (i.e. liver perfusion) due to a reduction in fatty liver, may result in a more pronounced increase in midazolam systemic plasma clearance value of 1.7^{34, 35}. For morbidly obese patients, the reduced hepatic CYP3A activity (1.5 reduced $CL_{int,H}$) may be compensated by an increase in hepatic blood flow in comparison to healthy volunteers resulting in similar plasma midazolam systemic plasma clearance value compared to healthy volunteers^{17, 36}. As such, both changes in CYP3A and liver blood flow and/or perfusion contribute to the overall effects observed in midazolam plasma clearance in morbidly obese and weight loss patients compared to healthy volunteers.

It seems that information on the hepatic blood flow and perfusion in patients after weight loss surgery is crucial to understand the results and to support the above described hypothesis that hepatic blood flow or perfusion improves after weight loss surgery. Also, for morbidly obese patients information on hepatic blood flow and perfusion is scarce. For this reason, we considered in our analysis different hepatic blood flow scenarios (Table 1, Figure 4), while a choice for any of these or other hepatic blood flow

scenarios cannot be justified. Scenario 1, in which the hepatic blood flow equation by Brown *et al.* was used, seems to lead to rather large values for morbidly obese patients ($Q_H = 3.7$ L/min at 144 kg) ²⁵. At first sight, scenario 2 seems more plausible for morbidly obese patients, as hepatic blood flow values are derived from the cardiac output function by Young *et al.* in which data of morbidly obese patients were included as well ²⁶, however the Q_H values for weight loss patients may be considered too low ($Q_H = 1.3$ L/min at 98 kg), while in healthy volunteers Q_H is generally considered to be 1.6 L/min ²⁵. Scenario 3, assuming a similar blood flow across all body weights, may not be so unrealistic considering the fact that the calculated plasma clearance values are in good agreement with actual results found in our earlier study (Figure 4) ^{8,9}. Future research should elucidate how hepatic blood flow is affected by morbid obesity and weight loss surgery to be able to further improve predictions on how CYP3A mediated hepatic drug clearance is affected.

Midazolam intrinsic gut wall clearance, $CL_{int,G}$, was low in both patient groups in comparison to results from healthy volunteers that were obtained using a similar semi-PBPK model (i.e. 0.0199 (35%) versus 0.45 (0.98-0.52) L/min, respectively) ¹⁵. As a result, the derived values for F_G were near 1 for both patient groups (Figure 4). In addition, a trend for a lower $CL_{int,G}$ for weight loss patients could be observed (Figure 3). This result may be attributed to the 75-150 cm bypass of relatively CYP3A rich initial part of the intestines, which is similar to the mechanism that may explain the increase in F_G for controlled release formulation for highly permeable CYP3A substrates ³⁷. However, the trend of lower $CL_{int,G}$ for weight loss patients was not statistically significant. This may in part be due to the high inter individual variability in $CL_{int,G}$ observed for both groups. For morbidly obese patients, the relatively low $CL_{int,G}$ estimate is in line with the increase in midazolam oral bioavailability (F_{total}) in comparison to healthy volunteers reported earlier (0.60 (13%) versus 0.28 (7%), $p < 0.01$) ¹⁷.

To further investigate the consequences of 1.5 times increased hepatic CYP3A intrinsic clearance for other drugs, the SimCYP simulator was used in which the 1.5 increase in hepatic CYP3A abundance in the morbidly obese population was mimicked. For the CYP3A substrates cyclosporine, alprazolam and triazolam plasma clearance was 1.30-1.41 times increased as opposed to 1.22 for midazolam. This difference in impact on plasma clearance between the drugs may be explained by the difference in extraction ratio of the substrates. Midazolam is considered an intermediate extraction ratio drug ($E_H = \sim 0.4$ ^{22, 23, 24}), while cyclosporine, alprazolam and triazolam are low extraction ratio drugs ($E_H = 0.05-0.25$ ^{38, 39, 40}). From these simulations, it can be concluded that the systemic plasma clearance of low extraction ratio CYP3A substrates is increased by at least 1.3 times after weight loss surgery, while, due to the lack of knowledge on how hepatic blood flow is affected by weight loss surgery, no definite conclusions can be drawn for CYP3A substrates with median and higher extraction ratios. Finally, these exploratory

extrapolations should be interpreted with caution, as it has been shown that the *in vivo* clearance of in CYP3A probes may correlate poorly⁴¹.

We conclude that a semi-PBPK model taking into account both gut wall and liver processes, adequately describes midazolam and CYP3A mediated 1-OH-midazolam concentrations after both oral and intravenous administration in morbidly obese patients before and after a weight loss surgery. Using this model it was revealed that in patients one year after weight loss surgery CYP3A hepatic intrinsic metabolizing capacity is recovered in comparison to morbidly obese patients before weight loss surgery, while CYP3A mediated gut wall intrinsic clearance seems to be lower.

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CONFLICTS OF INTEREST

None to declare

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SUPPLEMENTARY MATERIAL TO CHAPTER 7

Supplementary information 1

Table 1 Calculated values for F_G and F_H using literature values for intravenous midazolam clearance (CL_{iv}) and oral bioavailability (F_{total})

Population	CL_{iv} (L/min) (from ref.)	F_{total} (from ref.)	F_H (calculated)	F_G (calculated)
<i>Morbidly obese patients (n=20, 144 kg) ¹</i>				
$Q_{H,B} = 2.6$ L/min (scenario 1 ²)	0.385	0.56	0.78	0.72
$Q_{H,B} = 1.9$ L/min (scenario 2 ³)	0.385	0.56	0.69	0.81
$Q_{H,B} = 1.75$ L/min (scenario 3)	0.385	0.56	0.67	0.84
Mean	0.385	0.56	0.71 ± 0.06	0.79 ± 0.06
<i>Bariatric patients (n=18, 98 kg) ¹</i>				
$Q_{H,B} = 2.0$ L/min (scenario 1 ²)	0.647	0.56	0.50	1.13
$Q_{H,B} = 1.3$ L/min (scenario 2 ³)	0.647	0.56	0.25	2.26
$Q_{H,B} = 1.75$ L/min (scenario 3)	0.647	0.56	0.44	1.27
Mean	0.647	0.56	0.39 ± 0.13	1.55 ± 0.61
<i>Healthy volunteer studies (n=38) ^{4, 5, 6}</i>				
$Q_{H,B} = 1.6-1.9$ L/min from references	0.49 ± 0.04	0.31 ± 0.03	0.60 ± 0.06	0.52 ± 0.1
$Q_{H,B} = 1.6$ L/min (scenario 1 ²)	0.49 ± 0.04	0.31 ± 0.03	0.53	0.58
$Q_{H,B} = 1.2$ L/min (scenario 2 ³)	0.49 ± 0.04	0.31 ± 0.03	0.37	0.83
$Q_{H,B} = 1.75$ L/min (scenario 3)	0.49 ± 0.04	0.31 ± 0.03	0.57	0.54
Mean	0.49 ± 0.04	0.31 ± 0.03	0.52 ± 0.10	0.62 ± 0.14

Calculated F_G and F_H values based on literature clearance and oral bioavailability values

Total oral bioavailability of a drug, F_{total} , was defined as

$$F_{total} = F_a \cdot F_G \cdot F_H$$

in which, F_a is the fraction of drug absorbed into the gut wall and assumed to be equal to 1 for midazolam, F_G is the fraction of drug or metabolite escaping gut wall metabolism and F_H is the fraction of drug or metabolite escaping hepatic metabolism. Using the ‘well-stirred’ liver model the value for midazolam F_H can be derived from intravenous plasma clearance (CL_{iv})⁷, assuming negligible extrahepatic clearance:

$$F_H = 1 - \frac{CL_{iv}}{Q_{H,B} \times (C_B / C_P)}$$

in which $Q_{H,B}$ is the hepatic blood flow and C_B/C_P is the midazolam blood-to-plasma ratio (0.66)⁴. The value for $Q_{H,B}$ were determined using different models for cardiac output

and/or hepatic blood flow^{2, 3, 8}. For morbidly obese patients and bariatric patients the hepatic blood flow was calculated based on the median body weights, 144 kg and 98 kg.

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SUPPLEMENTARY INFORMATION 2

A QUASI-STEADY STATE SIMPLIFICATION OF A SEMI-PHYSIOLOGICAL ABSORPTION MODEL

The original model

A semi-physiological absorption model for the midazolam pharmacokinetics was originally described by Frechen *et al.*¹ with the following differential equations for gut wall, portal vein, liver, central, shallow peripheral and the deep peripheral compartments, respectively.

$$\begin{aligned}
 \frac{dA_{GW,mdz}}{dt} &= I(t) - \frac{Q_{villi}F_{G,mdz}A_{GW,mdz}}{V_{GW}} - \frac{Q_{villi}E_{G,mdz}A_{GW,mdz}}{V_{GW}} \\
 \frac{dA_{PV,mdz}}{dt} &= \frac{Q_{villi}F_{G,mdz}A_{GW,mdz}}{V_{GW}} + \frac{Q_{PV}A_{c,mdz}}{V_{c,mdz}} - \frac{Q_{PV}A_{PV,mdz}}{V_{PV}} \\
 \frac{dA_{H,mdz}}{dt} &= \frac{Q_{PV}A_{PV,mdz}}{V_{PV}} + \frac{Q_{HA}A_{c,mdz}}{V_{c,mdz}} - \frac{Q_H E_{H,mdz} A_{H,mdz}}{V_H} - \frac{Q_H F_{H,mdz} A_{H,mdz}}{V_H} \\
 \frac{dA_{c,mdz}}{dt} &= \frac{F_{H,mdz} Q_H A_{H,mdz}}{V_H} - \frac{(Q_{HA} + Q_{PV} + Q_{1,mdz} + Q_{2,mdz}) A_{c,mdz}}{V_{c,mdz}} + \frac{Q_{1,mdz} A_{p1,mdz}}{V_{p1,mdz}} + \frac{Q_{2,mdz} A_{p2,mdz}}{V_{p2,mdz}} \\
 \frac{dA_{p1,mdz}}{dt} &= \frac{Q_{1,mdz} A_{c,mdz}}{V_{c,mdz}} - \frac{Q_{1,mdz} A_{p1,mdz}}{V_{p1,mdz}} \\
 \frac{dA_{p2,mdz}}{dt} &= \frac{Q_{2,mdz} A_{c,mdz}}{V_{c,mdz}} - \frac{Q_{2,mdz} A_{p2,mdz}}{V_{p2,mdz}}
 \end{aligned}$$

Where A_x denote amounts in compartments x , V denote compartment volumes (physiological or empirical), Q denote blood flows or empirical inter-compartmental clearances and $I(t)$ is the time-dependent input function into the gut wall compartment.

The hepatic bioavailability was described with the well-stirred model of hepatic blood clearance:

$$F_{H,mdz} = \frac{Q_H}{Q_H + CL_{int,H,mdz} f_{u_{B,mdz}}}$$

Where $CL_{int,H,mdz}$ is the intrinsic hepatic clearance and $f_{u_{B,mdz}}$ is the midazolam unbound fraction in blood.

The intestinal bioavailability was described with the “Qgut” model, which inherits its structure from the well-stirred model of hepatic blood clearance¹:

$$F_{G,mdz} = \frac{Q_{GUT}}{Q_{GUT} + CL_{int,G,mdz}fu_{G,mdz}}$$

Where $CL_{int,G,mdz}$ is the intrinsic intestinal clearance and $fu_{B,mdz}$ is the midazolam unbound fraction in gut. The Q_{GUT} is a hybrid parameter of enterocytic villous blood flow and drug permeability CL_{perm} :

$$Q_{GUT} = \frac{Q_{villi}CL_{perm}}{Q_{villi} + CL_{perm}}$$

Furthermore, the metabolite pharmacokinetics was modeled with the following equations for the gut wall, portal vein, liver, central and peripheral metabolite compartments.

$$\begin{aligned} \frac{dA_{GW,met}}{dt} &= \frac{Q_{villi}E_{G,mdz}A_{GW,mdz}}{V_{GW}} - \frac{Q_{villi}E_{G,met}A_{GW,met}}{V_{GW}} - \frac{Q_{villi}F_{G,met}A_{GW,met}}{V_{GW}} \\ \frac{dA_{PV,met}}{dt} &= \frac{Q_{villi}F_{G,met}A_{GW,met}}{V_{GW}} + \frac{Q_{PV}A_{c,met}}{V_{c,met}} - \frac{Q_{PV}A_{PV,met}}{V_{PV}} \\ \frac{dA_{H,met}}{dt} &= \frac{Q_H E_{H,mdz} A_{H,mdz}}{V_H} + \frac{Q_{PV} A_{PV,met}}{V_{PV}} + \frac{Q_{HA} A_{c,met}}{V_{c,met}} - \frac{Q_H E_{H,met} A_{H,met}}{V_H} - \frac{Q_H F_{H,met} A_{H,met}}{V_H} \\ \frac{dA_{c,met}}{dt} &= \frac{F_{H,met} Q_H A_{H,met}}{V_H} - \frac{Q_{HA} + Q_{PV} + Q_{1,met}}{V_{c,met}} + \frac{Q_{1,met} A_{p1,met}}{V_{p1,met}} \\ \frac{dA_{p1,met}}{dt} &= \frac{Q_{1,met} A_{c,met}}{V_{c,met}} - \frac{Q_{1,met} A_{p1,met}}{V_{p1,met}} \end{aligned}$$

The original authors² reported that the choice of physiological compartment volumes (gut wall, portal vein and liver) had minimal influence on the parameter estimates and the objective function value of the model. Therefore, the volumes were fixed to one liter in the original analysis.

The organ volumes mainly represent an additional delay in the orally administered drug reaching the central compartments of the midazolam and 1-OH-metabolite. This can be readily seen from their respective equations. The organ volumes do not affect the bioavailability fractions, which already make use of the quasi-steadystate approximation by the use of the well-stirred models.

Therefore, the finding of the original authors that the volume of the physiological compartments has little effect on parameter estimates and the objective function, is an indication that the physiological compartments were only adding negligible delay for the drug to reach the central midazolam and 1-OH-midazolam compartments. Further, all of these physiological compartments are well-perfused, which further encourages the conclusion that the delay provided by them is negligible.

Therefore, as no observations are available from these physiological compartments, their main function is to explain which fraction of the dose ends up in the midazolam compartment, and which fraction ends up as a metabolite because of first-pass metabolism.

Model reduction by quasi-steady state approximation

Quasi-steady state approximation (QSSA) is a technique commonly used in systems biology³. It involves assuming that one of the compartments or states in the system exists in an equilibrium with regard to other compartments or states of the system; in other words, we set $dA_x/dt \approx 0$. Then, the amounts in compartment x can be calculated analytically, and this compartment can be omitted from the system of differential equations.

The first reduction was to assume quasi-steadystate in the gut wall, for both midazolam and the metabolite. For the parent, this leads to

$$\frac{dA_{GW}}{dt} = I(t) - \frac{Q_{villi}F_{G,mdz}A_{GW,mdz}}{V_{GW}} - \frac{Q_{villi}E_{G,mdz}A_{GW,mdz}}{V_{GW}} \approx 0$$

$$A_{GW,mdz} = \frac{I(t)}{Q_{villi}/V_{GW}}$$

And for the metabolite in the gut wall

$$\frac{dA_{GW,met}}{dt} = \frac{Q_{villi}E_{G,mdz}A_{GW,mdz}}{V_{GW}} - \frac{Q_{villi}E_{G,met}A_{GW,met}}{V_{GW}} - \frac{Q_{villi}F_{G,met}A_{GW,met}}{V_{GW}} \approx 0$$

$$A_{GW,met} = A_{GW,mdz}E_{GW,mdz}$$

Then, using these approximations for the gut wall drug amounts, we can calculate the amounts in the portal vein for the parent

$$\frac{dA_{PV,mdz}}{dt} = \frac{Q_{villi}F_{G,mdz}A_{GW,mdz}}{V_{GW}} + \frac{Q_{PV}A_{c,mdz}}{V_{c,mdz}} - \frac{Q_{PV}A_{PV,mdz}}{V_{PV}} \approx 0$$

$$A_{PV,mdz} = \frac{\frac{Q_{villi}}{V_{GW}}F_{G,mdz}A_{GW,mdz} + \frac{Q_{PV}}{V_{c,mdz}}A_{c,mdz}}{Q_{PV}/V_{PV}}$$

And for the metabolite in the portal vein

$$\frac{dA_{PV,met}}{dt} = \frac{Q_{villi}F_{G,met}A_{GW,met}}{V_{GW}} + \frac{Q_{PV}A_{c,met}}{V_{c,met}} - \frac{Q_{PV}A_{PV,met}}{V_{PV}} \approx 0$$

$$A_{PV,met} = \frac{\frac{Q_{villi}}{V_{GW}}F_{G,met}A_{GW,met} + \frac{Q_{PV}}{V_{c,met}}A_{c,met}}{Q_{PV}/V_{PV}}$$

With these solutions for portal vein compartment, we can calculate the quasi-steadystate amounts in the liver for the parent:

$$\begin{aligned}\frac{dA_{H,mdz}}{dt} &= \frac{Q_{PV}A_{PV,mdz}}{V_{PV}} + \frac{Q_{HA}A_{c,mdz}}{V_{c,mdz}} - \\ &\quad \frac{Q_H E_{H,mdz} A_{H,mdz}}{V_H} - \frac{Q_H F_{H,mdz} A_{H,mdz}}{V_H} \approx 0 \\ A_{H,mdz} &= \frac{\frac{Q_{HA}}{V_c} A_{c,mdz} + \frac{Q_{PV}}{V_{PV}} A_{PV,mdz}}{Q_H/V_H}\end{aligned}$$

And for the metabolite in the liver compartment:

$$\begin{aligned}\frac{dA_{H,met}}{dt} &= \frac{Q_H E_{H,mdz} A_{H,mdz}}{V_H} + \frac{Q_{PV} A_{PV,met}}{V_{PV}} + \frac{Q_{HA} A_{c,met}}{V_{c,met}} - \\ &\quad \frac{Q_H E_{H,met} A_{H,met}}{V_H} - \frac{Q_H F_{H,met} A_{H,met}}{V_H} \approx 0 \\ A_{H,met} &= \frac{\frac{Q_{HA}}{V_{c,met}} A_{c,met} + \frac{Q_{PV}}{V_{PV}} A_{PV,met} + \frac{Q_H}{V_H} E_{H,mdz} A_{H,mdz}}{Q_H/V_H}\end{aligned}$$

Finally, we can use these definitions to rewrite the original model in reduced form for the parent:

$$\begin{aligned}\frac{dA_{c,mdz}}{dt} &= \frac{F_H Q_H A_{H,mdz}}{V_H} - \frac{(Q_{HA} + Q_{PV} + Q_{1,mdz} + Q_{2,mdz}) A_{c,mdz}}{V_{c,mdz}} + \\ &\quad \frac{Q_{1,mdz} A_{p1,mdz}}{V_{p1,mdz}} + \frac{Q_{2,mdz} A_{p2,mdz}}{V_{p2,mdz}} \\ \frac{dA_{p1,mdz}}{dt} &= \frac{Q_{1,mdz} A_{c,mdz}}{V_{c,mdz}} - \frac{Q_{1,mdz} A_{p1,mdz}}{V_{p1,mdz}} \\ \frac{dA_{p2,mdz}}{dt} &= \frac{Q_{2,mdz} A_{c,mdz}}{V_{c,mdz}} - \frac{Q_{2,mdz} A_{p2,mdz}}{V_{p2,mdz}}\end{aligned}$$

And for the metabolite:

$$\begin{aligned}\frac{dA_{c,met}}{dt} &= \frac{F_H Q_H A_{H,met}}{V_H} - \frac{(Q_{HA} + Q_{PV} + Q_{1,met}) A_{c,met}}{V_{c,met}} + \\ &\quad \frac{Q_{1,met} A_{p1,met}}{V_{p1,met}} \\ \frac{dA_{p1,met}}{dt} &= \frac{Q_{1,met} A_{c,met}}{V_{c,met}} - \frac{Q_{1,met} A_{p1,met}}{V_{p1,met}}\end{aligned}$$

Model reduction into a compartmental model

It is possible to further reduce the model defined with the quasi-steady state approximations. First, using the defined approximations we can simplify that the midazolam concentration in the liver is

$$\begin{aligned}
A_{H,mdz} &= \frac{\frac{Q_{HA}}{V_{c,mdz}} A_{c,mdz} + \frac{Q_{PV}}{V_{PV}} A_{PV,mdz}}{Q_H/V_H} \\
&= \frac{\frac{Q_{HA}}{V_{c,mdz}} A_{c,mdz} + \frac{Q_{PV}}{V_{PV}} \left(\frac{l(t)F_{G,mdz} + \frac{Q_{PV}}{V_{c,mdz}} A_{c,mdz}}{Q_{PV}/V_{PV}} \right)}{Q_H/V_H} \\
&= \frac{l(t)F_{G,mdz} + Q_H A_{c,mdz}/V_{c,mdz}}{Q_H/V_H}
\end{aligned}$$

And the metabolite concentration in the liver is

$$\begin{aligned}
A_{H,m} &= \frac{\frac{Q_{HA}}{V_{c,met}} A_{c,met} + \frac{Q_{PV}}{V_{PV}} A_{PV,met} + \frac{Q_H}{V_H} A_{H,mdz} E_{H,mdz}}{Q_H/V_H} \\
&= \frac{\frac{Q_{HA}}{V_{c,met}} A_{c,met} + \frac{Q_{PV}}{V_{PV}} \left(\frac{l(t)E_{G,mdz} F_{G,met} + \frac{Q_{PV}}{V_{c,met}} A_{c,met}}{Q_{PV}/V_{PV}} \right)}{Q_H/V_H} + \\
&\quad \frac{\frac{Q_H}{V_H} \left(\frac{l(t)F_{G,mdz} + Q_H A_{c,mdz}/V_{c,mdz}}{Q_H/V_H} \right) E_{H,mdz}}{Q_H/V_H} \\
&= \frac{l(t)E_{G,mdz} F_{G,met} + l(t)F_{G,mdz} E_{H,mdz}}{Q_H/V_H} + \\
&\quad \frac{A_{c,met} V_H}{V_{c,m}} + \frac{A_{c,mdz} E_{H,mdz} V_H}{V_c}
\end{aligned}$$

Substituting the quasi-steady state approximations, we get the following expressions for the parent and metabolite central compartment differential equations:

$$\begin{aligned}
\frac{dA_{c,mdz}}{dt} &= \frac{F_H Q_H \left(\frac{l(t)F_{G,mdz} + Q_H A_{c,mdz}/V_{c,mdz}}{Q_H/V_H} \right)}{V_H} - \\
&\quad \frac{(Q_{HA} + Q_{PV} + Q_{1,mdz} + Q_{2,mdz}) A_{c,mdz}}{V_{c,mdz}} + \\
&\quad \frac{Q_{1,mdz} A_{p1,mdz}}{V_{p1,mdz}} + \frac{Q_{2,mdz} A_{p2,mdz}}{V_{p2,mdz}} \\
\frac{dA_{c,met}}{dt} &= \frac{F_{H,met} Q_H A_{H,met}}{V_H} - \frac{Q_{HA} + Q_{PV} + Q_{1,met}}{V_{c,met}} A_{c,met} + \frac{Q_{1,met} A_{p1,met}}{V_{p1,met}}
\end{aligned}$$

Keeping in mind that

$$\begin{aligned}
&\frac{F_{H,mdz} Q_H \left(\frac{l(t)F_{G,mdz} + Q_H A_{c,mdz}/V_{c,mdz}}{Q_H/V_H} \right)}{V_H} - \frac{Q_H A_{c,mdz}}{V_{c,mdz}} \\
&= F_{G,mdz} F_{H,mdz} l(t) + \frac{F_{H,mdz} Q_H A_{c,mdz}}{V_{c,mdz}} - \frac{Q_H A_{c,mdz}}{V_{c,mdz}} \\
&= F_{G,mdz} F_{H,mdz} l(t) - \frac{CL_{B,mdz} A_{c,mdz}}{V_{c,mdz}}
\end{aligned}$$

And

$$\begin{aligned}
& \frac{F_{H,met} Q_H \left(\frac{I(t) E_{G,mdz} F_{G,met} + I(t) F_{G,mdz} E_{H,mdz}}{Q_H / V_H} \right)}{V_H} + \\
& \frac{F_{H,met} Q_H \left(\frac{A_{c,met} V_H}{V_{c,met}} + \frac{A_{c,mdz} E_{H,mdz} V_H}{V_{c,mdz}} \right)}{V_H} - \frac{Q_H A_{c,met}}{V_{c,met}} \\
& = F_{H,met} Q_H \left(\frac{I(t) E_{G,mdz} F_{G,met} + I(t) F_{G,mdz} E_{H,mdz}}{Q_H} \right) + \\
& F_{H,met} Q_H \left(\frac{A_{c,met}}{V_{c,met}} + \frac{A_{c,mdz} E_{H,mdz}}{V_{c,mdz}} \right) - \frac{Q_H A_{c,met}}{V_{c,met}} \\
& = I(t) E_{G,mdz} F_{G,met} F_{H,met} + I(t) F_{G,mdz} E_{H,mdz} F_{H,met} + \\
& \frac{A_{c,met} F_{H,met} Q_H}{V_{c,met}} - \frac{Q_H A_{c,met}}{V_{c,met}} + \frac{A_{c,mdz} E_{H,mdz} F_{H,met} Q_H}{V_{c,mdz}} \\
& = (E_{G,mdz} F_{G,met} + F_{G,mdz} E_{H,mdz}) I(t) F_{H,met} \\
& - \frac{CL_{B,met} A_{c,met}}{V_{c,met}} + \frac{A_{c,mdz} CL_{B,mdz} F_{H,met}}{V_{c,mdz}}
\end{aligned}$$

It is possible to rewrite the original model without most of the blood flows; the only blood flows necessary are either Q_{GUT} or Q_{Hr} , in order to differentiate between the hepatic and intestinal first-pass metabolism. Further, as the volumes (V) of the gut wall, portal vein and liver cancel themselves out, fixing them to arbitrary values will have no effect on model prediction after quasi-steady state approximations.

$$\begin{aligned}
\frac{dA_{c,mdz}}{dt} &= F_{G,mdz} F_{H,mdz} I(t) - \frac{(CL_{B,mdz} + Q_{1,mdz} + Q_{2,mdz}) A_{c,mdz}}{V_{c,mdz}} + \\
& \frac{Q_{1,mdz} A_{p1,mdz}}{V_{p1,mdz}} + \frac{Q_{2,mdz} A_{p2,mdz}}{V_{p2,mdz}} \\
\frac{dA_{p1,mdz}}{dt} &= \frac{Q_{1,mdz} A_{c,mdz}}{V_{c,mdz}} - \frac{Q_{1,mdz} A_{p1,mdz}}{V_{p1,mdz}} \\
\frac{dA_{p2,mdz}}{dt} &= \frac{Q_{2,mdz} A_{c,mdz}}{V_{c,mdz}} - \frac{Q_{2,mdz} A_{p2,mdz}}{V_{p2,mdz}} \\
\frac{dA_{c,met}}{dt} &= (E_{G,mdz} F_{G,met} + F_{G,mdz} E_{H,mdz}) I(t) F_{H,met} - \\
& \frac{CL_{B,met} A_{c,met}}{V_{c,met}} + \frac{A_{c,mdz} CL_{B,mdz} F_{H,met}}{V_{c,mdz}} - \\
& \frac{Q_{1,met} A_{c,met}}{V_{c,met}} + \frac{Q_{1,met} A_{p1,met}}{V_{p1,met}} \\
\frac{dA_{p1,met}}{dt} &= \frac{Q_{1,met} A_{c,met}}{V_{c,met}} - \frac{Q_{1,met} A_{p1,met}}{V_{p1,met}}
\end{aligned}$$

Comparison by simulation

A simulation was conducted based on parameter estimates of an intermediate model, to verify that the quasi-steady state approximation does not cause significant bias in the results. For the purposes of the simulation, the parameters outlined in Table 2 were used. The simulation results showed that the quasi-steady state approximation produces only a minimal discrepancy to the predicted midazolam and 1-OH-midazolam concentrations (Figure 1).

Table 2 Physiological and model-related parameters used in the simulation

PK Parameter	Value
$I(t)$	$0.15 \cdot e^{-0.02 \text{ min}^{-1} t} \text{ mg/min}$
Intrinsic gut wall clearance	0.02 L/min
Intrinsic gut wall clearance (1-OH)	0.0 L/min
Distribution volume (central)	40 L
Intrinsic hepatic clearance	25 L/min
Distribution volume (shallow peripheral)	100 L
Inter-compartmental clearance (shallow)	0.9 L/min
Distribution volume (deep peripheral)	50 L
Inter-compartmental clearance (deep)	0.2 L/min
Distribution volume (central, 1-OH)	65 L
Intrinsic hepatic clearance (1-OH)	3 L/min
Distribution volume (peripheral, 1-OH)	40 L
Inter-compartmental clearance (1-OH)	0.3 L/min
Physiological constant	Value
CL_{perm}	0.1766 L/min
$fu_{B,mdz}$	0.033
$fu_{G,mdz}$	1
$fu_{B,met}$	1
$fu_{G,met}$	1

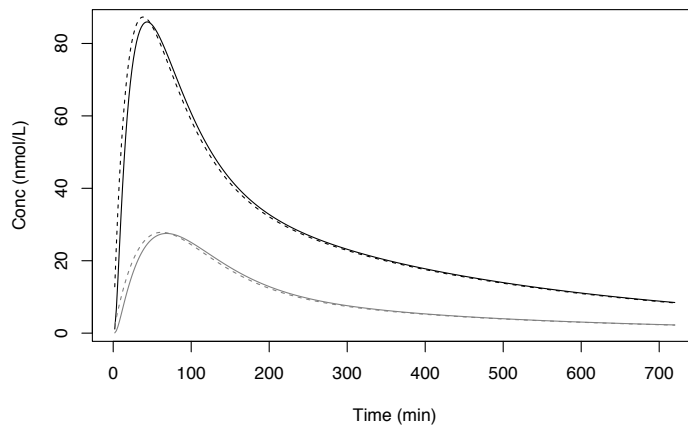


Figure 1 A comparison of simulated concentrations from the original model (solid lines) and the reduced model (dashed lines). Black lines indicate midazolam concentrations and grey lines indicate 1-OH-midazolam concentrations.

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2. Frechen, S. et al. A semiphysiological population pharmacokinetic model for dynamic inhibition of liver and gut wall cytochrome P450 3A by voriconazole. *Clin. Pharmacokinet.* 2013; 52, 763-781.
3. Ingalls, B. P. *Mathematical Modeling in Systems Biology: An Introduction.* MIT Press, 2013.

SUPPLEMENTARY INFORMATION 3

NONMEM MODEL CODE

```
$PROBLEM MDZ+1-OH in Morbidly Obese and Bariatric pts
$INPUT ID TIME AMT RATE DROP DV PLAS CMT MDV BQL TAD TADI OBES TBW LOSS LBW
IBW BMI WHR AGE SEX LOQ IV OCC
$DATA data.csv IGNORE=@
$SUBROUTINES ADVAN6 TOL=6
$MODEL
COMP=(PODOSE,DEFDOSE) ;1
COMP=(CENTRAL) ;2 Midazolam blood conc.
COMP=(PERIP) ;3
COMP=(PER2) ;4
COMP=(1OHCENT) ;5 1-OH blood conc.
COMP=(PER1OH) ;6
COMP=(TRANSIT1) ;7
COMP=(TRANSIT2) ;8
COMP=(TRANSIT3) ;9
COMP=(TRANSIT4) ;10
COMP=(TRANSIT5) ;11

$PK
; parent (MDZ)

IF(OCC.EQ.1) TVV5= THETA(1)*(1+THETA(21)*(TBW-127))
IF(OCC.EQ.2) TVV5= THETA(1)
V5= TVV5*EXP(ETA(1))

IF(OBES.EQ.1) TVQ= THETA(2)
IF(OBES.EQ.2) TVQ= THETA(2)*THETA(24)
Q= TVQ*EXP(ETA(2)) ; clearance blood to periph comp (L/min)

IF(OBES.EQ.1) TVV6=THETA(3)*(TBW/127)**THETA(20)
IF(OBES.EQ.2) TVV6=THETA(3)
V6= TVV6*EXP(ETA(3)) ; volume periph. comp (L)

IF(OBES.EQ.1) TVKA=THETA(4)
```

IF(OBES.EQ.2) TVKA=THETA(22)
 KA= TVKA*EXP(ETA(11)) ; min-1
 KTR=KA

F1= THETA(5)*EXP(ETA(4)) ; FA (fraction absorbed)
 ALAG1= THETA(6)*EXP(ETA(5)) ; lag time (min)
 V12=THETA(17)*V6

Q12=THETA(18)
 VPV= 1 ; portal vein compartment fixed to 1 L.

IF(OBES.EQ.1) TCH= THETA(7)
 IF(OBES.EQ.2) TCH= THETA(23)
 CLH= TCH*EXP(ETA(6)) ; intrinsic hepatic clearance (unbound)
 FUB= THETA(8) ; fraction unbound in blood

QH= 3.75*TBW**0.75/60 ; in L/min (according to Brown et al. 1997)
 QPV= 0.75*QH ; portal vein blood flow (75% from blood flow of liver) from Williams et al. 1989
 QHA= 0.25*QH ; hepatic artery blood flow (25% from liver blood flow) Williams et al. 1989
 VH= 1 ; fixed to 1 L.

CLG= THETA(9)*EXP(ETA(7)) ; intrinsic intestinal clearance (unbound)

FUG= 1 ; fixed to 1, acc to Yang et al. 2007
 QIN= 0.4*QH ; intestinal blood flow, acc to Williams et al. 1989
 QMU= 0.8*QIN ; mucosa blood flow, according to Yang et al. 2007
 QVI= 0.6*QMU ; villous blood flow, acc to Yang et al. 2007
 VGW= 1 ; Volume of Gut wall fixed to 1 L.

; 1-OH-MDZ parameters

VMET=THETA(10)*EXP(ETA(8)) ; volume of central 1-OH cmt
 CLHM=THETA(11)*EXP(ETA(9)) ; intrinsic hepatic clearance of 1-OH
 FUBM=THETA(12) ; fraction unbound in blood of 1-OH
 CLGM=THETA(13)*EXP(ETA(10)) ; intrinsic gut clearance of 1-OH
 FUGM=THETA(14) ; fraction unbound in gut of 1-OH
 VPER=THETA(15)
 QPER=THETA(16)

BP=0.66 ; blood:plasma ratio
 CLPL= (QH*FUB*CLH)/(QH+(FUB*CLH/BP)) ; calculated plasma clearance

; hepatic extraction parent

$$EH = (CLH * FUB) / (QH + (CLH * FUB))$$

$$FH = 1 - EH$$

; gutwall extraction, QG= "Qgut" parent

CLP= THETA(19) ; permeability 10.6 L/h = 0.1766 L/min (Yang et al. 2007)

$$QGUT = (QVI * CLP) / (QVI + CLP)$$

$$EG = (CLG * FUG) / (QGUT + (CLG * FUG))$$

$$FG = 1 - EG$$

; hepatic extraction 1-OH-MDZ

$$EHM = (CLHM * FUBM) / (QH + (CLHM * FUBM))$$

$$FHM = 1 - EHM$$

; gutwall extraction, QG= "Qgut" and equals Qvilli (QVI) in this case

$$EGM = (CLGM * FUGM) / (QVI + (CLGM * FUGM))$$

$$FGM = 1 - EGM$$

$$S2 = V5$$

$$S5 = VMET$$

$$K17 = KA$$

$$K78 = KTR$$

$$K89 = KTR$$

$$K910 = KTR$$

$$K1011 = KTR$$

$$K112 = KTR$$

$$K56 = Q/V5$$

$$K65 = Q/V6$$

$$K512 = Q12/V5$$

$$K125 = Q12/V12$$

$$K1011 = QPER/VMET$$

$$K1110 = QPER/VPER$$

$$FA = F1$$

\$DES

$$AGUTW = KTR * A(11) / (QVI / VGW)$$

$$APV = ((QVI / VGW) * AGUTW * FG + QPV / V5 * A(2)) / (QPV / VPV)$$

$$AH = (QHA / V5 * A(2) + QPV / VPV * APV) / (QH / VH)$$

$$AGUTWM = (1 - FG) * AGUTW$$

$$APVM = ((QVI / VGW) * AGUTWM * FGM + QPV / VMET * A(5)) / (QPV / VPV)$$

$AHM = (QHA/VMET * A(5) + QPV/VPV * APVM + QH/VH * AH * EH) / (QH/VH)$

$DADT(1) = -K17 * A(1)$

$DADT(2) = FH * (QH/VH) * AH - (QHA/V5) * A(2) - (QPV/V5) * A(2) - K56 * A(2) + K65 * A(3) - K512 * A(2) + K125 * A(4)$; central

$DADT(3) = K56 * A(2) - K65 * A(3)$; 1st periperal cmt

$DADT(4) = K512 * A(2) - K125 * A(4)$; 2nd periph

$DADT(5) = FHM * (QH/VH) * AHM - (QPV/VMET) * A(5) - (QHA/VMET) * A(5) - K1011 * A(5) + K1110 * A(6)$; centralIM

$DADT(6) = K1011 * A(5) - K1110 * A(6)$; peri 1-OH cmt

$DADT(7) = K17 * A(1) - KTR * A(7)$

$DADT(8) = KTR * A(7) - KTR * A(8)$

$DADT(9) = KTR * A(8) - KTR * A(9)$

$DADT(10) = KTR * A(9) - KTR * A(10)$

$DADT(11) = KTR * A(10) - KTR * A(11)$

\$ERROR

COM1=0

IF (OBES.EQ.1) COM1=1

COM2=0

IF (OBES.EQ.2) COM2=1

IPRED=F ; individual prediction

$Y1 = IPRED * (1 + ERR(1))$; Morbidly obese patients

$Y2 = IPRED * (1 + ERR(2))$; Bariatric surgery patients

$Y = Y1 * COM1 + Y2 * COM2$

IRES=DV-IPRED ; individual residual

DEL=0

IF (IPRED.EQ.0) DEL=1

$IWRES = (1 - DEL) * IRES / (IPRED + DEL)$;

\$THETA

; midazolam

(0, 40) ;1, V5 (L)

(0, 1.3) ;2, Q

(0, 100) ;3, V6 (L)

(0, 0.1) ;4, KA MO

1 FIX ;5, FA (fraction absorbed fixed to 1)
 0 FIX ;6, ALAG1
 (0, 20) ;7, CLH MO
 0.033 FIX ;8, Fraction unbound in blood (FUB)
 (0, 0.01) ;9, CLG intrinsic gut wall clearance
 (0, 65) ;10, VMET Volume of metabolite 1-OH-MDZ
 (0, 3) ;11, CLHM intrinsic hepatic clearance of 1-OH
 0.106 FIX ;12, FUBM Fraction unbound in blood 1-OH (Mandema et al.)
 (0, 5) ;13, CLGM intrinsic gut clearance of 1-OH
 1 FIX ;14, FUGM fraction unbound in gut of metabolite 1-OH
 (0, 40) ;15, VPER
 (0, 0.3) ;16, QPER
 (0, 7) ;17, V12
 (0, 0.5) ;18, Q12
 0.1766 FIX ;19, CLperm
 3.93 FIX ;20, TBW pow V6
 0.0435 FIX ;21, TBW lin V5
 (0, 0.2) ;22, KA BA
 (0, 30) ;23, CLH BA
 3.22 FIX ;24, fQ BA

\$OMEGA ; perc. standrd dev. van interind.var(eta)

0.2 ; 1, V5

0 FIX ; 2, Q

0.5 ; 3, V6

0 FIX ; 4, F1

0 FIX ; 5, ALAG1

0.05 ; 6, CLH

0.9 ; 7, CLG

0 FIX ; 8, VMET Volume of metabolite 1-OH-MDZ

0 FIX ; 9, CLHM

0 FIX ; 10, CLGM

0.2 ; 11, KA

\$SIGMA ; residuele (error/epsilon)

0.07 ; MO

0.04 ; BA

\$EST SIGDIG=2 MAXEVAL=9999 PRINT=5 NOABORT METHOD=1 INTERACTION POSTHOC

MSFO=RUN5.nmv

\$COV COMP

