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The impact of obesity on the pharmacokinetics of drugs in adolescents and adults

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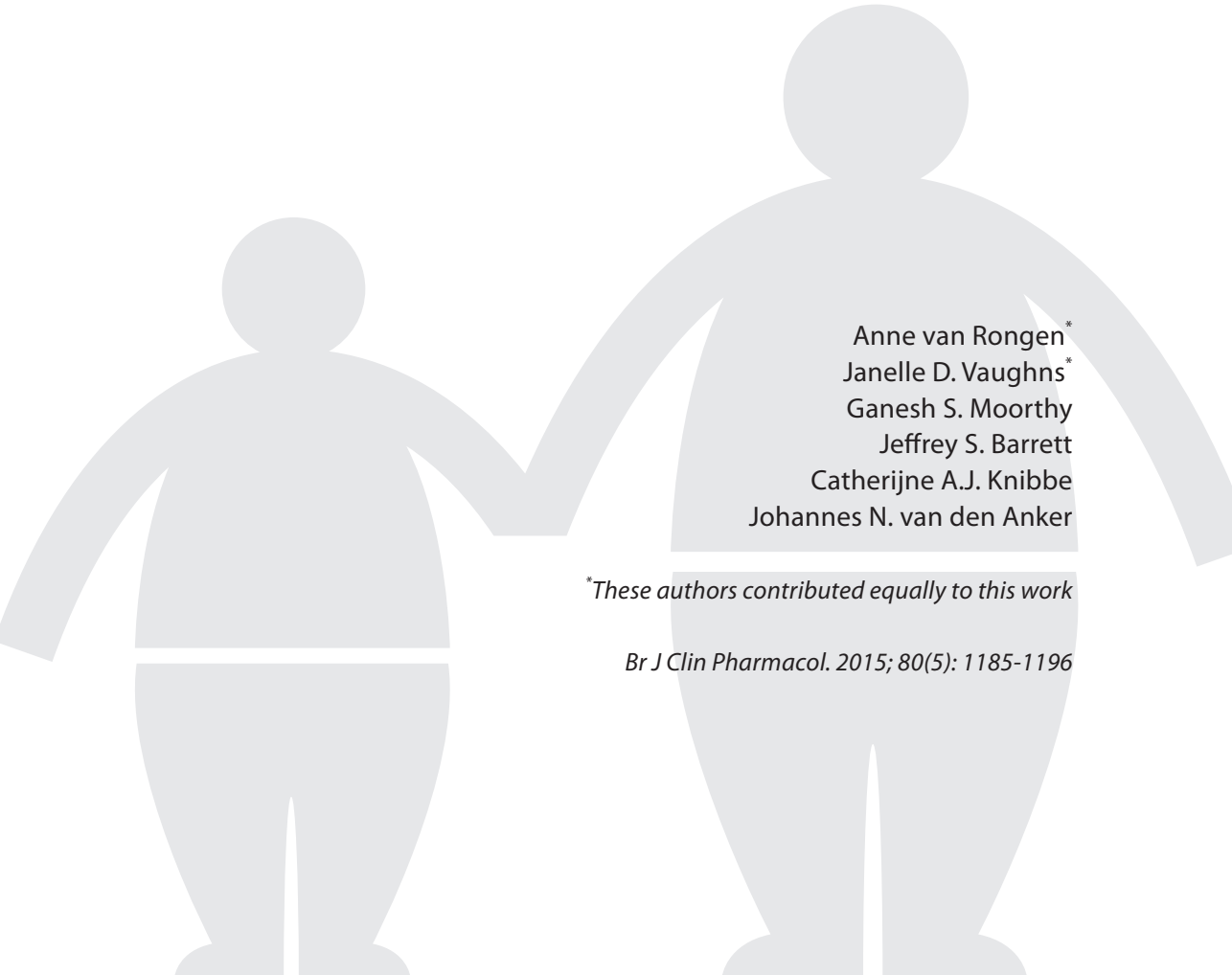
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Chapter 4

Population pharmacokinetics of midazolam and its metabolites in overweight and obese adolescents



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ABSTRACT

Aim

In view of the increasing prevalence of obesity in adolescents, the aim of this study was to determine the pharmacokinetics of the CYP3A substrate midazolam and its metabolites in overweight and obese adolescents.

Methods

Overweight (BMI for age $\geq 85^{\text{th}}$ percentile) and obese (BMI for age $\geq 95^{\text{th}}$ percentile) adolescents undergoing surgery received 2 or 3 mg intravenous midazolam as a sedative drug pre-operatively. Blood samples were collected until 6 or 8 h post-dose. Population pharmacokinetic modelling and systematic covariate analysis was performed using NONMEM 7.2.

Results

Nineteen overweight and obese patients with a mean body weight of 102.7 kg (62 - 149.8 kg), a mean BMI of 36.1 kg/m² (24.8 - 55 kg/m²), and a mean age of 15.9 years (range 12.5 - 18.9 years) were included. In the model for midazolam and metabolites, total body weight was not of influence on clearance (0.66 L/min (RSE 8.3%)), while peripheral volume of distribution of midazolam (154 L (11.2%)), increased substantially with total body weight ($P < 0.001$). The increase in peripheral volume could be explained by excess body weight (WT_{excess}) instead of body weight related to growth ($WT_{\text{for age and length}}$).

Conclusions

The pharmacokinetics of midazolam and metabolites in overweight and obese adolescents show a marked increase in peripheral volume of distribution and a lack of influence on clearance. The findings may imply a need for a higher initial infusion rate upon initiation of a continuous infusion in obese adolescents.

INTRODUCTION

To date, the prevalence rates of overweight (BMI for age $\geq$ 85th percentile) and obese (BMI for age $\geq$ 95th percentile) adolescents have increased substantially ¹. According to the National Health and Nutrition Examination Survey in the United States in 2011-2012, 34.5% of the adolescents (12-19 years) were overweight and 20.5% obese ¹. Worldwide prevalence rates of overweight and obese adolescents are also high, exceeding 24%, for instance, in Spain, Italy, Australia, Saudi Arabia, Brazil and Argentina ². Due to the co-morbid disease state accompanying early obesity, these patients may require frequent drug administration, including anaesthetics for orthopaedic or bariatric surgery ^{3,4}.

Despite increasing numbers of obese adolescents, there is a paucity of dosing guidelines for this population due to the limited number of available pharmacokinetic and/or pharmacodynamic studies in this special patient population ⁵⁻⁷. A guide for dosing in obese individuals is particularly important for adolescents, as paediatric dosing guidelines are typically expressed in mg/kg, which may lead to overdosing in overweight or obese adolescents ⁸. However, the evaluation of the influence of (over)weight on the pharmacokinetics in obese children is complicated by the interrelation between growth, age and obesity, i.e. with increasing age, body weight may increase as a result of growth, obesity or both ⁸. An important question in this respect is therefore if obese adolescents should be dosed based on their total body weight and/or how their state of (over)weight should be considered for dosing ⁸.

Midazolam is a commonly used lipophilic benzodiazepine for preoperative sedation in paediatric anaesthesia because of its potent sedative, amnesic and anxiolytic properties. Midazolam is considered one of the best CYP3A probe drugs, since it is extensively metabolized by CYP3A ⁹ to its major metabolite 1-OH-midazolam and rapidly excreted into urine as its glucuronide conjugate ¹⁰. While it has previously been suggested that CYP3A clearance in obese patients is reduced as compared with non-obese patients ^{11,12}, two studies on the pharmacokinetics of midazolam in obese adult patients have shown no alteration in midazolam clearance as compared with non-obese adults ^{13,14}. As the pharmacokinetics of CYP3A metabolized drugs have not yet been studied in obese adolescents, the aim of this study was to evaluate the pharmacokinetics of midazolam and its major metabolites 1-OH-midazolam and 1-OH-midazolam glucuronide in overweight and obese adolescents.

METHODS

Patients

Overweight and obese adolescents from 12 to 18 years of age undergoing general surgery (such as orthopaedics, tonsillectomy, bariatric surgery) with an American Society Anaesthesiologist (ASA) physical status of I, II or III were considered for participation in the study. Overweight and obesity were classified as BMI for age between 85th and 95th percentile and as BMI for age $\geq$ 95th percentile, respectively ¹⁵. Patients were excluded if they were pregnant, had prior exposure to a benzodiazepine within an 8 h period, had a known hypersensitivity to any benzodiazepine, a history of central nervous system dysfunction or active upper airway disease, a liver or renal disease, or if they were treated with drugs known to affect CYP3A, such as certain anti-epileptics, imidazole derivatives, macrolides, corticosteroids, and grapefruit juice. Before participation, parents and patients provided written informed consent and assent, respectively. The study was approved by the Institutional Review Board (IRB) at Children's National Medical Center in Washington DC (IRB protocol no 4718) and was conducted in accordance with the principles of the Declaration of Helsinki.

Study design

In this prospective observational study patients received a single intravenous bolus dose of 2 or 3 mg midazolam a few minutes before they were taken to the operating room. Blood samples were collected at T=0, (5), 15, 30 min and 1, 2, 4, 6 and occasionally 8 h. Blood samples were collected in lithium heparin tubes and centrifuged at 1500 g for 15 min at 4°C and plasma was stored at -80°C until analysis. Midazolam, 1-OH-midazolam and 1-OH-midazolam glucuronide concentrations were measured using high performance liquid chromatography with electro-spray ionization tandem mass spectrometry ¹⁶. Since authentic 1-OH-midazolam glucuronide standards are not available, samples were hydrolysed with β -glucuronidase under optimized conditions. The difference in concentration between total and free 1-OH-midazolam metabolites provided the concentration of conjugated 1-OH-midazolam metabolites. Assay was linear over 0.5 to 1,000 ng/mL with the limits of quantitation (LOQ) of 0.5 ng/mL for midazolam and 1-OH-midazolam. Intra and interday accuracy and precision were within 85-115% and 15% (CV), respectively. Recoveries were $> 70\%$ for all analytes, with matrix effects less than 15% over six batches of plasma. Stability in plasma and extracts was sufficient under assay conditions.

Population pharmacokinetic analysis and internal model validation

The pharmacokinetic data were analysed using non-linear mixed effects modelling with NONMEM (version 7.2; ICON Development Solutions, Hanover, MD, USA) ¹⁷ and

Pirana (2.8.1)¹⁸, R (3.0.1)¹⁹, Xpose (4.5.0)¹⁸ and Psn (3.6.2)¹⁸ were used to evaluate and visualize the data. The first order conditional estimation method was used for model development. Discrimination between different models was guided by comparison of the objective function value (OFV, i.e. -2 log likelihood (-2LL)) between nested models. A *P* value of < 0.05, representing a decrease of 3.84 in OFV for one degree of freedom, was considered statistically significant. In addition, goodness-of-fit plots for midazolam, 1-OH-midazolam and 1-OH-midazolam glucuronide (observed vs. individual predicted concentrations, observed vs. population predicted concentrations, conditional weighted residuals vs. time after dose, and conditional weighted residuals vs. population predicted concentrations plots) were used for diagnostic purposes. Furthermore, precision of parameter estimates, the correlation matrix and visual improvement in the individual plots were used to evaluate the model. Pharmacokinetic models incorporating two or three compartments for midazolam and one or two compartments for the metabolites were tested. In the model it was assumed that the volume of distribution of 1-OH-midazolam is 0.9 times the volume of distribution of midazolam²⁰. Interindividual variability (IIV) was assumed to follow a log normal distribution. Residual variability was tested using proportional, additive or a combined proportional and additive error models for midazolam and metabolites. Concentrations were expressed as µmol/L using the molecular weights of midazolam, 1-OH-midazolam and 1-OH-midazolam glucuronide (325.77, 341.77 and 517.9, respectively). For midazolam, 1-OH-midazolam and 1-OH-midazolam glucuronide no samples (0%), 11 samples (8.5%) and one sample (0.8%) were below the LOQ, respectively, and were removed from the analysis^{21,22}. For internal model evaluation, a bootstrap resampling method using 1000 replicates and prediction-corrected visual predictive checks (pcVPCs)²³ stratified for midazolam, 1-OH-midazolam and 1-OH-midazolam glucuronide using 1000 simulated datasets of individuals from the original dataset were used.

Covariate model

Tested covariates were total body weight (TBW), BMI, BMI z-score²⁴, lean body weight (LBW) according to the equation of Janmahasatian et al.²⁵, LBW according to the equation of Peters et al.²⁶, LBW according to Foster et al.²⁷, age, gender, race and type of surgery. A BMI z-score is the number of standard deviation away from the mean based on growth charts of the Center for Disease Control (CDC)^{24,28,29}. Covariates were plotted independently against the eta estimates of the pharmacokinetic parameters to visualize potential relations. Continuous covariates were tested using linear and power equations (Equation 1 and 2, respectively):

$$P_i = P_p \times (1 + Y \times (COV - COV_{median})) \quad (\text{Eq. 1})$$

$$P_i = P_p \times (COV/COV_{median})^X \quad (\text{Eq. 2})$$

where P_i and P_p represent individual and population parameter estimates, respectively, COV represents the covariate, COV_{median} represents the median value of the covariate for the population, Y represents a correlation factor between the population pharmacokinetic parameter and the change in covariate value for a linear function and X represent the exponent for a power function. The categorical covariates, gender, race and type of surgery, were examined by calculating a separate parameter for each category of the covariate.

Potential covariates were univariately entered into the model and statistically tested by use of the objective function value (OFV). In addition, if applicable, it was evaluated whether the interindividual variability (IIV, η) of the parameter concerned decreased upon inclusion of the covariate on the parameter and whether the trend in the η of the parameter vs. the covariate involved was resolved. When more than one significant covariate was identified, the covariate-adjusted model with the largest decrease in the OFV was chosen as a basis to explore sequentially the influence of additional covariates with the use of the same criteria. Finally, after forward inclusion ($P < 0.01$), a backward exclusion procedure was applied to justify the inclusion of a covariate ($P < 0.001$). The choice of the final covariate model was further evaluated as discussed in the population pharmacokinetic analysis and internal model validation section.

(Over)weight covariate model

To analyse further the influence of (over)weight on the pharmacokinetics of midazolam, an (over)weight covariate model was tested for the parameters for which total body weight proved a covariate given the above mentioned criteria. In this covariate model, the total body weight of each individual patient was considered to be composed of two parts: body weight related to growth (body weight for age and length, $WT_{\text{for age and length}}$) and excess body weight (WT_{excess}). This (over)weight covariate model is adapted from an exploratory model reported by Bartelink et al. ³⁰, who used $WT_{\text{for age}}$ instead of $WT_{\text{for age and length}}$ with the latter being more relevant for adolescents ³¹. For each individual patient of our study $WT_{\text{for age and length}}$ and WT_{excess} were calculated using Equation 3 and 4, respectively:

$$WT_{\text{for age and length}} = BMI_{\text{without overweight}} \times \text{length}^2 \quad (\text{Eq. 3})$$

$$WT_{\text{excess}} = TBW - WT_{\text{for age and length}} \quad (\text{Eq. 4})$$

in which $BMI_{\text{without overweight}}$ is the BMI derived from the BMI for age CDC growth chart at a BMI z score of 0 together with the age of the patient ²⁸ and TBW is the total body weight of the patient.

$WT_{\text{for age and length}}$ and WT_{excess} were both plotted independently against the eta estimate of the pharmacokinetic parameter of interest to visualize the relation. Equation 1 or 2 were used to quantify the relation of $WT_{\text{for age and length}}$ and WT_{excess} with the pharmacokinetic parameter at the same criteria for model selection as discussed under the covariate model section.

Simulations

The final population pharmacokinetic model was used to simulate (population predictions without IIV or residual variability) concentration vs. time curves for three patients from the dataset, i.e. a median individual of 105 kg and two extremes of the dataset (i.e. 62 and 149 kg) upon a 0.05 mg/kg intravenous bolus dose, a 0.1 mg/kg/h continuous infusion, a fixed 2 mg intravenous bolus dose, a fixed 3.5 mg/h continuous infusion and a fixed 3.5 mg/h continuous infusion with an increased initial infusion rate.

RESULTS

Patients and data

Twenty patients were enrolled in the study. One patient was excluded from analysis, because this patient proved to have a normal weight for his length (misreported length in the medical status). In total nineteen overweight and obese patients were included in the analysis with a total of 129 midazolam plasma samples, 118 1-OH-midazolam and 128 1-OH-midazolam glucuronide. Three patients received a 3 mg bolus dose of midazolam and 16 patients received a 2 mg bolus dose. A summary of all patient characteristics are presented in Table 1.

Table 1 Demographic parameters of 19 overweight and obese adolescents.

	Overweight and obese adolescents (n=19)
Female/male	13/6
Overweight/obese	3/16
Age (years)	15.9 ± 1.6 (12.5-18.9)
Body weight (kg)	102.7 ± 24.9 (62-149.8)
BMI (kg/m ²)	36.1 ± 8.1 (24.8-55)
BMI z-score	2.2 ± 0.39 (1.5-2.7)
LBW (kg) eq. Janmahasatian et al. ²⁵	57.3 ± 11.1 (39.8-74.4)
LBW (kg) eq. Foster et al. ²⁷	58.4 ± 12.5 (39.7-78.9)
LBW(kg) eq. Peters et al. ²⁶	66.4 ± 11.8 (46.0-84.4)

Values are expressed as mean ± SD (range) unless specified otherwise.

BMI= body mass index, eq.= equation, LBW= lean body weight.

Population pharmacokinetic model and internal model evaluation

A two compartment model for midazolam, a one compartment model for 1-OH-midazolam and a two compartment model for 1-OH-midazolam glucuronide best described the data (Figure 1). Residual variability was best described by three proportional error models for the midazolam, 1-OH-midazolam and 1-OH-midazolam glucuronide concentrations. Table 2 shows the parameter estimates of the base model without covariates.

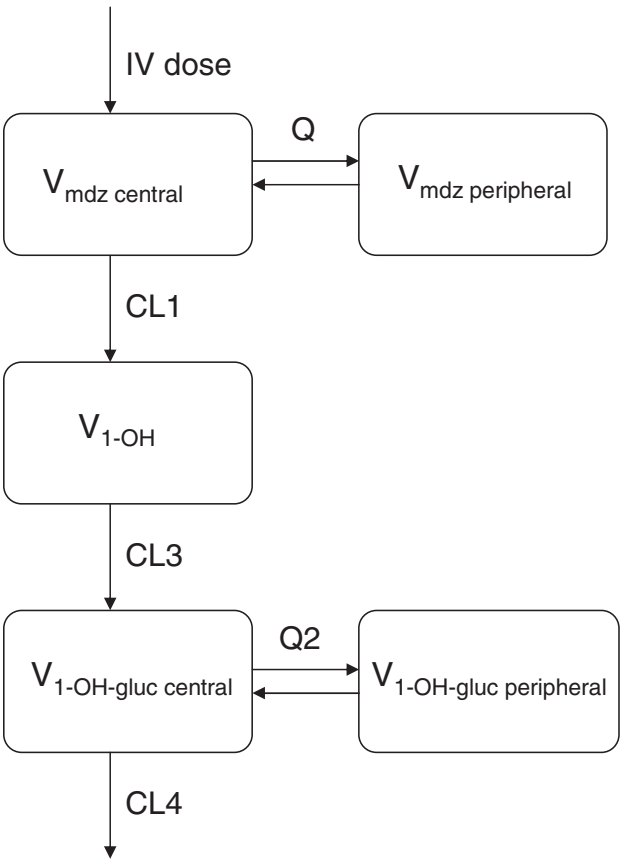


Figure 1 Schematic illustration of the population pharmacokinetic midazolam model. CL1= clearance of midazolam to 1-OH-midazolam, CL3= clearance of 1-OH-midazolam to 1-OH-midazolam glucuronide, CL4= clearance of 1-OH-midazolam glucuronide, MDZ= midazolam, 1-OH= 1-OH-midazolam, 1-OH-gluc= 1-OH-midazolam glucuronide, Q= inter-compartmental clearance from the central compartment of midazolam to the peripheral compartment of midazolam, Q2= inter-compartmental clearance from the central compartment of 1-OH-midazolam glucuronide to the peripheral compartment of 1-OH-midazolam glucuronide, V= volume of distribution.

Table 2 Population pharmacokinetic parameters of the base and final pharmacokinetic model for midazolam in overweight and obese adolescents and results of bootstrap analysis.

Parameter	Base model (RSE%) [shrinkage%]	Final model (RSE%) [shrinkage%]	Bootstrap (95% confidence interval)
Midazolam			
CL1 (L/min)	0.66 (10.2)	0.66 (8.3)	0.66 (0.52-0.75)
V _{mdz central} (L)	39.8 (8.6)	39.8 (8.3)	39.13 (33.28-46.52)
V _{mdz peripheral} (L)	141 (9.9)	-	-
V _{mdz peripheral} = V _{104.7 kg} × (TBW/104.7) ^x			
V _{104.7 kg}	-	154 (11.2)	154.7 (119.89-237.22)
X	-	1.68 (12.1)	1.65 (0.9-2.63)
Q (L/min)	1.19 (10.8)	1.18 (15.6)	1.21 (0.90-1.59)
1-OH-midazolam			
CL3 (L/min)	1.86 (14.5)	1.85 (9.3)	1.85 (1.46-2.30)
1-OH-midazolam glucuronide			
V _{1-OH-gluc central} (L)	4.13 (14.5)	4.05 (17.5)	4.06 (1.39-5.97)
V _{1-OH-gluc peripheral} (L)	16 (16.6)	15.9 (9.5)	15.9 (11.57- 20.03)
Q2 (L/min)	0.48 (16.4)	0.49 (23.9)	0.50 (0.26- 0.78)
CL4 (L/min)	0.42 (8.4)	0.42 (6.4)	0.42 (0.36-0.48)
Interindividual variability (%)			
CL1	24.2 (19) [6]	23.7 (25) [6]	21.8 (9.0-33.1)
V _{mdz central} = V _{1-OH}	30.2 (18) [10]	30.5 (14.4) [10]	28.8 (17.9-39.9)
V _{mdz peripheral}	53.1 (19.6) [3]	30.2 (32.6) [13]	30.5 (4.1-45.9)
Q	40.2 (19.9) [6]	39.5 (18.8) [7]	36.5 (17.6-56.9)
CL3	27.6 (44.3) [6]	26.7 (20) [6]	24.5 (13.0- 37.3)
Residual variability (%)			
Proportional error MDZ	26.7 (19) [10]	26.5 (14) [9]	26.5 (21.4-35.3)
Proportional error 1-OH	25.9 (9) [11]	26.0 (13) [11]	25.6 (19.6-31.2)
Proportional error 1-OH-gluc	23.3 (3) [8]	23.4 (7) [7]	22.3 (19.0-25.8)
OFV (-2LL)	-3501	-3517	-3565 (-3783-, -3302)

CL1= clearance of midazolam to 1-OH-midazolam, CL3= clearance of 1-OH-midazolam to 1-OH-midazolam glucuronide, CL4= clearance of 1-OH-midazolam glucuronide, MDZ= midazolam, 1-OH= 1-OH-midazolam, 1-OH-gluc= 1-OH-midazolam glucuronide, RSE= relative standard error, TBW= total body weight, Q= inter-compartmental clearance from the central compartment of midazolam to the peripheral compartment of midazolam, Q2= inter-compartmental clearance from the central compartment of 1-OH-midazolam glucuronide to the peripheral compartment of 1-OH-midazolam glucuronide, V= volume of distribution, V_{104.7 kg}= peripheral volume of distribution of a 104.7 kg patient (median weight).

In the covariate analysis, a significant influence of total body weight (TBW) was found for peripheral volume of distribution of midazolam, with a power function best describing the data ($P < 0.001$, -16 OFV). After inclusion of TBW as a power function for peripheral volume of distribution, the trend in the eta value of peripheral volume of distribution disappeared and no residual trend was observed (Figure 2). This is also reflected by the

reduction of interindividual variability associated with peripheral volume of distribution (53.1% to 30.2%, Table 2). The Empirical Bayes estimates (EBEs) for peripheral volume of distribution of the base model and the estimated power function of the final model are shown in Supplementary Figure 1.

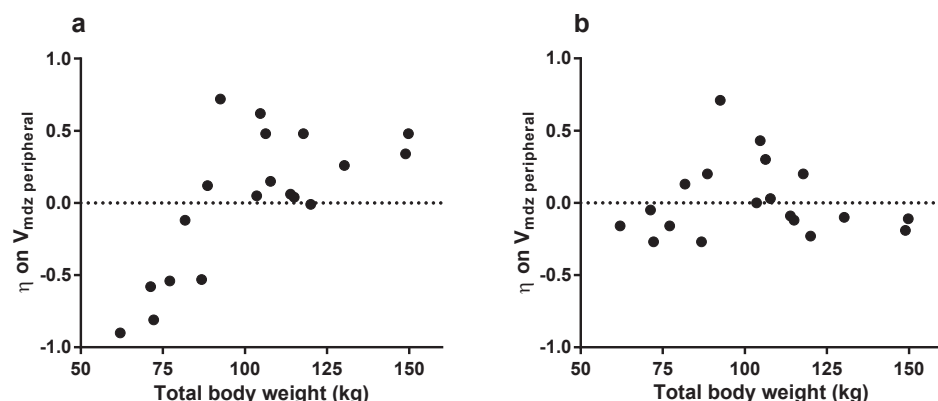


Figure 2 Interindividual random effects associated with peripheral volume of distribution of midazolam [η on $V_{mdz_peripheral}$] vs. total body weight (TBW) for the base model (a) and the final model (b).

A positive trend between clearance of midazolam to 1-OH-midazolam (CL1) and TBW or LBW using the equation of Peters et al.²⁶ was found. However none of the covariates achieved the criteria of the backward deletion step of the covariate analysis and parameters were not estimated with adequate precision. Similarly, for clearance of 1-OH-midazolam to 1-OH-midazolam glucuronide (CL3) a positive trend was found for TBW, but also did not achieve the criteria of the backward deletion step of the covariate analyses. Age did not show a statistically significant influence on any of the pharmacokinetic parameters ($P > 0.05$).

To differentiate between the influence of $WT_{for\ age\ and\ length}$ and WT_{excess} an (over)weight covariate model was also tested for peripheral volume of distribution. No trend was observed between $WT_{for\ age\ and\ length}$ and peripheral volume of distribution of midazolam (Figure 3a). However a positive trend was observed between WT_{excess} and peripheral volume of distribution which was best described by a power function ($P < 0.001$, -17 OFV) (Figure 3b). Since the OFV of this (over)weight covariate model was not significantly different from the final model (3518 vs. 3517, $P > 0.05$), this model was only used to illustrate that the increase in peripheral volume of distribution is probably caused by WT_{excess} of these adolescents.

The final model parameters are summarized in Table 2. Observed vs. individual predicted concentrations and observed vs. population predicted concentrations for midazolam, 1-OH-midazolam, and 1-OH-midazolam glucuronide are shown in Figure 4.

The bootstrap analysis confirmed the results of the model as the parameter estimates and eta estimates were within 6% and 14%, respectively of those obtained within the original dataset (Table 2). In addition, prediction-corrected VPCs (pcVPCs) for midazolam, 1-OH-midazolam and 1-OH-midazolam glucuronide indicated good predictive performance with good agreement between observed data and model simulated confidence intervals for the median, 2.5th and 97.5th percentiles (Figure 5).

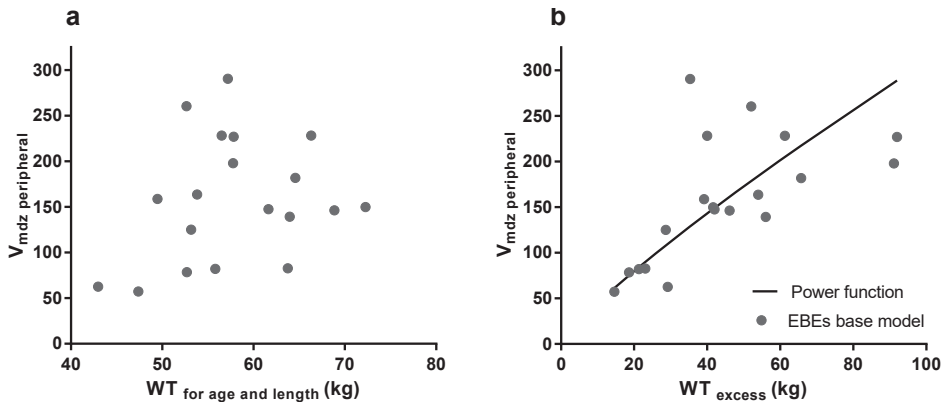


Figure 3 Empirical Bayes estimates (EBEs) for peripheral volume of distribution of midazolam ($V_{mdz_peripheral}$) vs. body weight related to growth ($WT_{for\ age\ and\ length}$) (a) and vs. excess body weight (WT_{excess}) with a power relation between peripheral volume of distribution of midazolam and WT_{excess} (b).

Simulations

Figure 6 shows midazolam concentrations after a 0.05 mg/kg intravenous bolus dose, a 0.1 mg/kg/h continuous infusion, a fixed 2 mg intravenous bolus dose and a fixed 3.5 mg/h continuous infusion in three representative patients (62, 105 and 145 kg). After administration of a mg/kg based bolus dose and a mg/kg continuous infusion, midazolam concentrations were substantially higher in individuals with a larger body weight (Figure 6a, 6b). Moreover, the time to steady-state concentration was increased in individuals with a larger body weight (Figure 6b). For the fixed intravenous bolus dose simulations there were only minor differences between the three patients (Figure 6c). After a fixed continuous infusion (Figure 6d) midazolam steady-state concentrations were at the same level for the three typical patients but were reached at a later time point with increasing body weight. In a 62 kg adolescent, steady-state will be reached after 19 h, while this is 37 h for a 105 kg adolescent and 64 h for a 149 kg adolescent (Figure 6d). In addition, after discontinuing the continuous infusion, midazolam concentrations decreased more slowly in heavier adolescents (Figure 6d).

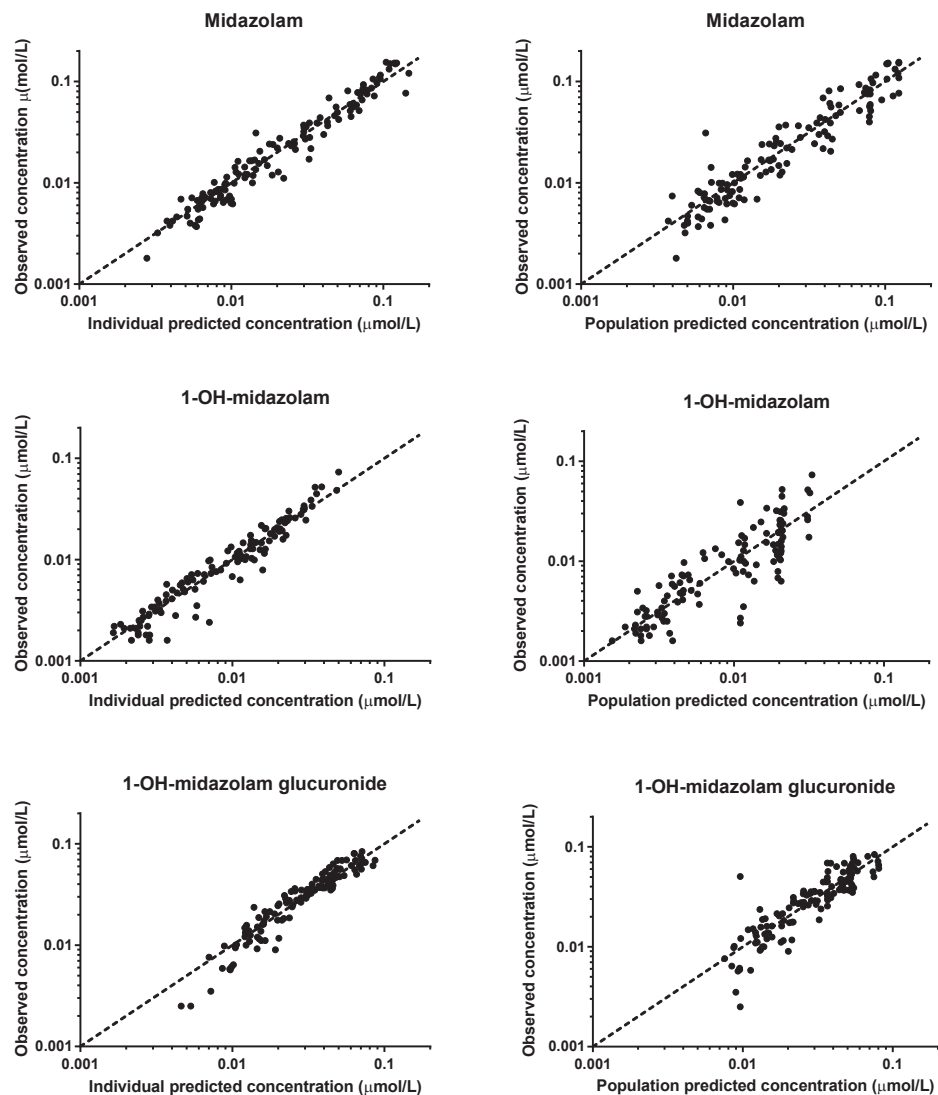


Figure 4 Observed vs. individual predicted concentrations and observed vs. population predicted concentrations of midazolam (upper panel), 1-OH-midazolam (middle panel) and 1-OH-midazolam glucuronide (lower panel).

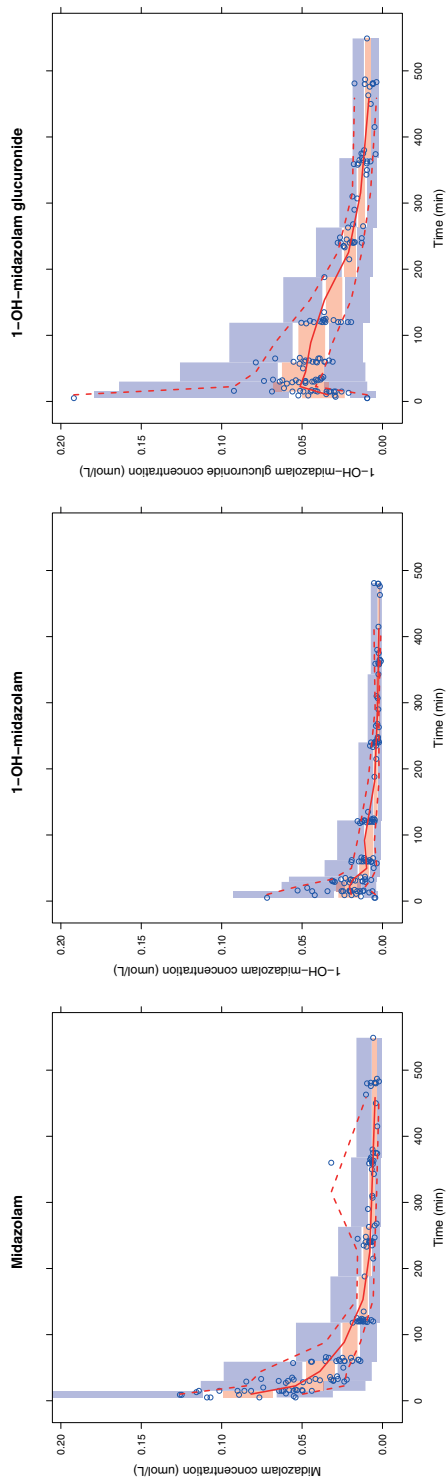


Figure 5 Prediction-corrected visual predictive checks of the final model for midazolam, 1-OH-midazolam and 1-OH-midazolam glucuronide. Observed concentrations are shown as blue circles with solid, lower and upper dashed red lines showing the median, 2.5th and 97.5th percentiles of the observed data, respectively. The shaded areas represent 95% confidence intervals for the model predicted median, 2.5th, 97th percentiles constructed from 1000 simulated datasets of individuals from the original dataset.

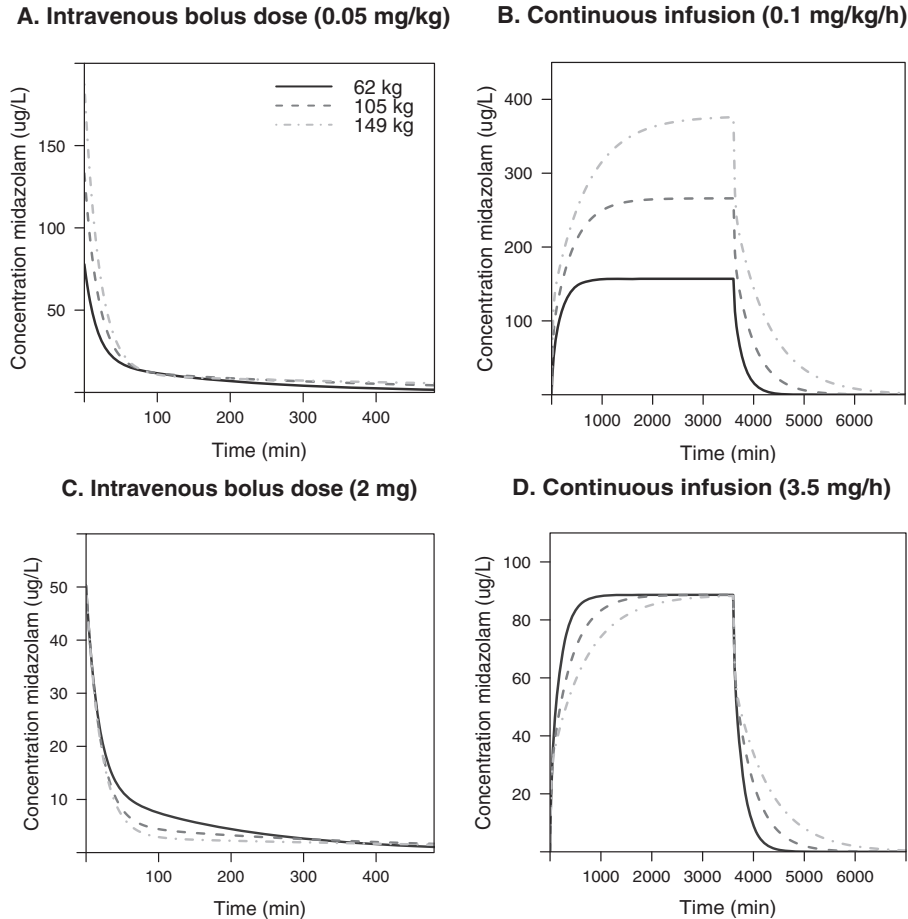


Figure 6 Population predicted midazolam concentrations over time in three overweight and obese adolescents (62, 105 and 149 kg) after a 0.05 mg/kg intravenous bolus dose (a), a 0.1 mg/kg/h continuous infusion (b), a fixed 2 mg intravenous bolus dose (c) and a fixed 3.5 mg/h continuous infusion (d).

DISCUSSION

As there is no information on the influence of overweight and obesity on the pharmacokinetics of CYP3A metabolized drugs in adolescents, this study aimed to evaluate the pharmacokinetics of the CYP3A substrate midazolam and its metabolites in overweight and obese adolescents. The results of this study show that the clearance of midazolam or its metabolites does not change with increasing body weight, but that peripheral volume of distribution of midazolam increases with total body weight according to a power function. Moreover this study shows that this increase can be explained by WT_{excess} in these adolescents instead of an increase in $WT_{\text{for age and length}}$ (Figure 3).

In this study, we could not identify an influence of total body weight on midazolam clearance, even though a wide range in body weight of overweight and obese adolescents was included in the study (62 -150 kg). Only a positive trend of midazolam clearance and total body weight or LBW (equation of Peters et al.²⁶) was identified, but this trend was not large enough for inclusion in the final covariate model. Our results are in agreement with the literature, as both Greenblatt et al. and Brill et al. also reported no difference in clearance values of midazolam between obese and non-obese patients^{13,14}. These studies were, however, conducted in adults and not in adolescents. Compared with literature values of non-obese adolescents, the value for clearance we report in overweight and obese adolescents (0.66 L/min, 0.39 L/h/kg) seems very similar. Reed et al. reported a mean clearance value normalized for body weight of 0.56 ± 0.23 L/h/kg in non-obese adolescents, which corresponds to 0.57 L/min (mean body weight of 62 ± 11.3 kg, mean age of 15.4 ± 0.2 years)³². In addition, Mandema et al. reported a mean clearance value of 0.52 ± 0.31 L/min (0.45 L/h/kg) for young non-obese adults (mean age of 22 ± 1 years and mean body weight of 69 ± 6 kg)²⁰. Although our finding of a lack of influence of obesity on midazolam clearance is consistent with the literature, it is not consistent with previous findings that with increasing body weight CYP3A clearance will be lower due to a decreased CYP3A enzyme activity upon the chronic inflammatory status in obese individuals^{11,12}. According to Brill et al. this lack of decrease in overall clearance can be explained by the fact that the relative reduction in CYP3A activity per unit of liver in obese patients is counteracted by a higher liver volume, resulting in a similar absolute hepatic CYP3A metabolizing capacity with increasing body weights¹³. Given these considerations, it seems that the modest (and non-significant) increase in clearance in our study, most likely results from an influence of weight due to growth, even though this cannot be further analysed due to limitations as the small sample size, lack of data of non-obese adolescents and small age range.

The increase we report in peripheral volume of distribution can in our opinion be explained by an increase in adipose tissue with increasing body weight, especially since midazolam is a lipophilic drug (log P of 2.5). However, Jain et al. concluded that changes in volume of distribution cannot be predicted on the basis of lipophilicity alone³³. They showed, based on an overview of the ratios of volume of distribution of various drugs in obese vs. non-obese individuals normalized with body weight, that for lipophilic drugs these ratios were increased, reduced or remained unchanged³³. Although volume of distribution is difficult to predict, the increase in peripheral volume of distribution of midazolam with total body weight we report here is consistent with the results of Greenblatt et al. and Brill et al., who also found a large increase in volume of distribution of midazolam with increasing body weight in morbidly obese adults^{13,14}. Since the evaluation of pharmacokinetic data in obese children may be complicated because of the interrelation between growth, age and obesity⁸, we considered total body weight to

consist of two parts, $WT_{\text{for age and length}}$ and WT_{excess} . The rationale for this subanalysis is that the influence of 1 kg of excess weight in children and adolescents on a pharmacokinetic parameter such as volume of distribution may not be equal to 1 kg of weight due to growth ($WT_{\text{for age and length}}$). In this additional (over)weight covariate model, we identified that the increase in peripheral volume of distribution of midazolam in this population of adolescents is explained by WT_{excess} and not by $WT_{\text{for age and length}}$. This positive trend between peripheral volume of distribution and WT_{excess} was best described by a power function (Figure 3) and follows the same trend as for TBW and peripheral volume of distribution (Supplementary Figure 1). Compared with the literature for non-obese adolescents, our value for volume of distribution of 181 L (central + peripheral volume of distribution) was somewhat higher even though it was quite similar when expressed per kg (1.8 L/kg). Reed et al. found for non-obese adolescents a volume of distribution normalized for body weight of 2.0 ± 0.7 L/kg corresponding to an absolute volume of distribution of 124 L (mean body weight of 62.0 ± 11.3 kg)³². In a study in eight non-obese young adults (age of 22 ± 1 years) a volume of distribution of 60 L (0.87 L/kg (mean body weight 69 ± 6 kg)) was reported²⁰. As such, it seems that volume of distribution of midazolam is higher in obese adolescents compared to non-obese counterparts.

The midazolam dose simulations on the basis of the final pharmacokinetic model illustrate the clinical relevance of the findings reported in the current study. When midazolam is administered upon a mg/kg basis, midazolam concentrations are substantially higher in individuals with a larger body weight (Figure 6a and 6b). It can be concluded that in overweight and obese adolescents dosing based on mg/kg for midazolam should be discouraged and that instead a fixed dose is preferred (Figure 6c and 6d). In case a continuous infusion is initiated, the time to reach steady-state concentrations is more than three times increased in an adolescent of 149 kg in comparison with an adolescent of 62 kg (Figure 6b and 6d), which is due to the increased peripheral volume of distribution. Therefore, a higher initial continuous infusion rate can be considered for obese adolescents to decrease the time to steady-state (Figure 7, Table 3). Figure 7 illustrates the resulting concentrations upon an increased initial infusion rate in obese adolescents: i.e. 10 mg/h for 1 h and 5 mg/h for 1 h for an overweight child (62 kg simulation), 10 mg/h for 1 h, 7.5 mg/h for 1 h and 5 mg/h for 3 h for an obese child (105 kg simulation) and 10 mg/h for 1 h, 7.5 mg/h for 2 h and 5 mg/h for 8 h for a morbidly obese child (149 kg simulation) (Figure 7, Table 3). The results of these simulations also show that upon a fixed intravenous bolus dose (Figure 6c) no difference in the midazolam concentrations are expected between various body weights. An example of a dosing scheme for an intravenous bolus dose and for a continuous infusion with initial increased infusion rates in overweight and obese adolescents is summarized at Table 3. Note that the intravenous bolus dose of 2 mg and the maintenance dose of the continuous infusion of 3.5 mg/h are an example of a chosen dose and can be adapted to clinical needs or effect.

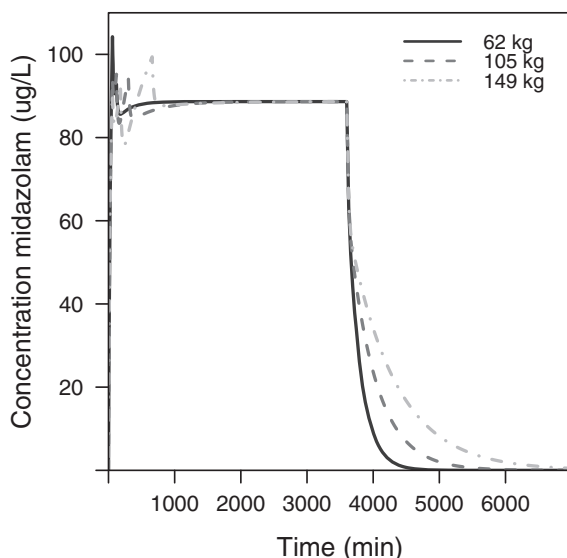


Figure 7 Population predicted midazolam concentrations over time in three overweight and obese adolescents (62, 105 and 149 kg) after a fixed 3.5 mg/h continuous infusion which is preceded by an increased initial infusion rate (see Table 3 for details of these increased initial infusion rates before the fixed 3.5 mg/h continuous infusion, i.e. for 62 kg: 10 mg/h for 1 h, 5 mg/h for 1 h, for 105 kg: 10 mg/h for 1 h, 7.5 mg/h for 1 h, 5 mg/h for 3 h, for 149 kg: 10 mg/h for 1 h, 7.5 mg/h for 2 h, 5 mg/h for 8 h).

The predictions, particularly for the peak concentrations, should be interpreted with care. In our final model, total body weight was not a significant covariate for central volume of distribution, even though a small positive trend was observed. This lack of significance may be due to variation in collection times of the first study sample (range of 5 - 29 min). To conclude definitively that there is no influence of weight on the central volume of distribution, all study subjects should have an early time point (± 5 min). Another limitation of the study is that no non-obese adolescents were included in the study, precluding a head to head comparison with overweight and obese adolescents. Finally, even though for body weight, a good stratification and distribution (62 - 145 kg) was reached during recruitment of the patients in the study, for age, more adolescents with an age < 14 years and an age of > 17 years should have been included in the analysis to test age accurately as a covariate.

Since the number of pharmacokinetic and/or pharmacodynamic studies in obese children and adolescents are limited⁵⁻⁷, more studies should be performed in this special population. For future data analysis in obese children and adolescents, we suggest to use our proposed (over)weight covariate model for proper evaluation of the exact influence of weight resulting from growth, obesity and age. In addition, a wide range of body sizes should be studied, which should be stratified by body weight, BMI and age¹². The inclusion of non-obese adolescents is also highly recommended, as this would put parameter estimates of the obese population into perspective and results in an even wider range of body sizes¹². Further recommendations for pharmacokinetic modelling in the obese population are described by van Rongen et al.¹².

Table 3 Example of a dosing scheme for overweight and obese adolescents aiming for similar midazolam concentrations in all individuals. Concentration – time profiles for individuals weighing 62, 105 and 149 kg given in this table are depicted in Figure 6c for the intravenous bolus dose and Figure 7 for the continuous infusion preceded by an increased initial infusion rate.

Body weight	Intravenous bolus dose*	Continuous infusion*
60 kg	2 mg	1 st hour
		2 nd hour
		3 rd and following hours
75 kg	2 mg	10 mg/h
		5 mg/h
		3.5 mg/h (fixed)
90 kg	2 mg	10 mg/h
		7.5 mg/h
		5 mg/h
		3.5 mg/h (fixed)
105 kg	2 mg	10 mg/h
		7.5 mg/h
		5 mg/h
		3.5 mg/h (fixed)
120 kg	2 mg	10 mg/h
		7.5 mg/h
		5 mg/h
		3.5 mg/h (fixed)
135 kg	2 mg	10 mg/h
		7.5 mg/h
		5 mg/h
		3.5 mg/h (fixed)
149 kg	2 mg	10 mg/h
		7.5 mg/h
		5 mg/h
		3.5 mg/h (fixed)

*NB: The intravenous bolus dose of 2 mg and the maintenance dose of the continuous infusion of 3.5 mg/h are an example of a chosen dose and can be adapted to clinical needs or effect.

In conclusion, this study represents a very unique dataset, since it is the first study evaluating the influence of overweight and obesity on the pharmacokinetics of the CYP3A substrate midazolam in adolescents. We have shown that midazolam clearance did not change with body weight in overweight and obese adolescents, but that the peripheral volume of distribution substantially increased with body weight. This increase will result in a prolonged time to reach steady-state concentrations when midazolam is given as a continuous infusion to adolescents with increasing body weight, which can be captured by an initial higher infusion rate. The increase in peripheral volume of distribution in obese individuals can be explained by WT_{excess} and not to $WT_{\text{for age and length}}$. We conclude that in overweight and obese adolescents dosing based on mg/kg for midazolam should be discouraged, and that instead a fixed dose can be used in this population. Future studies should not only focus on obese adolescents, but also on obese children, since age will play an even more important role for this group. In addi-

tion to intravenous midazolam, oral administration of midazolam in obese children and adolescents should also be investigated, since CYP3A is not only present in the liver, but also in the intestines and will influence the oral bioavailability of midazolam¹³.

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COMPETING INTERESTS

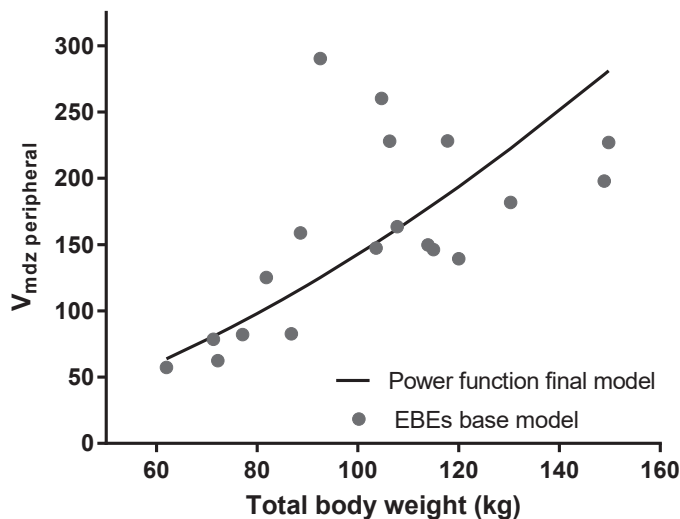
All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

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SUPPLEMENTARY MATERIAL



Supplementary Figure 1: Empirical Bayes estimates (EBEs) (dots) for peripheral volume of distribution of midazolam ($V_{mdz\ peripheral}$) vs. total body weight (TBW) in 19 overweight and obese adolescents of the base pharmacokinetic model with increase between peripheral volume of distribution with TBW according to the power function of the final model (line).