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Concepts and applications for evidence-based dosing in morbidly obese patients before and after weight loss surgery

Margreke J.E. Brill

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Concepts and applications for evidence-based dosing in morbidly obese patients before and after weight loss surgery

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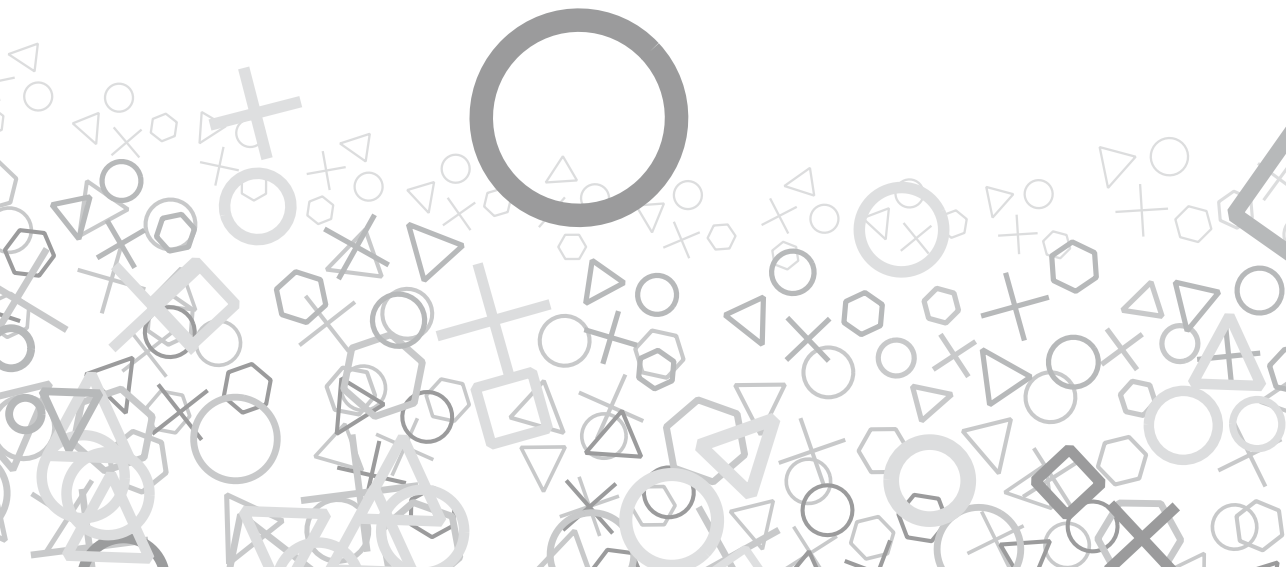
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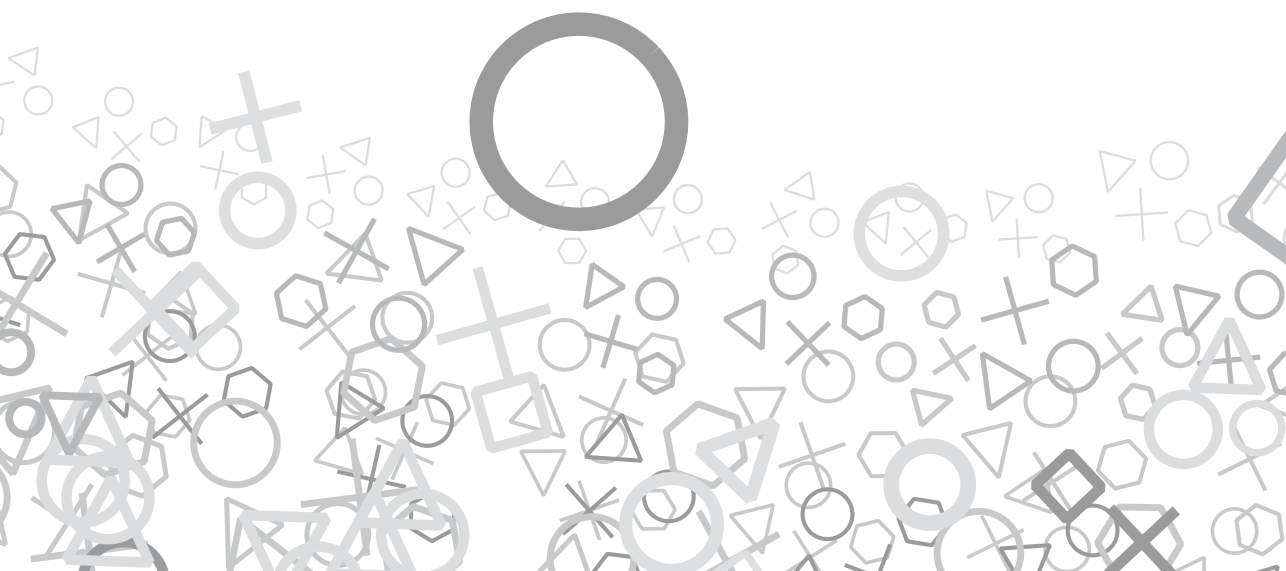
General introduction and background





CHAPTER 1

Introduction, scope and
outline of the investigations



MORBID OBESITY

The prevalence of obesity (body mass index, BMI > 30 kg/m²) and morbid obesity (BMI > 40 kg/m²) is increasing across the globe^{1,2}. Mexico and the US seem to deal with the highest obesity prevalence rates among adults, 32% and 35% respectively^{1,3}, while the estimated obesity prevalence rates for 2013 show that >50% of the women in Kuwait, Libya and Qatar are obese⁴. The average prevalence of obesity in Europe is estimated between 18-21% in 2013. In the Netherlands approximately 35% of the population is overweight (BMI > 25 kg/m²) and 13% obese⁵. Numbers of morbid obesity in Europe are lacking, but are estimated to range between 1-7% depending on the country⁵⁻⁸. A global analysis of overweight and obesity prevalence rates suggests that in developed countries the increasing obesity trend has attenuated over the past 8 years, while in the developing world the trend seems to continue to increase⁴.

With obesity and morbid obesity, physiologic parameters may alter. These patients are reported to have a lower percentage of fat free mass and water relative to their total body weight⁹⁻¹⁰, while absolute values of cardiac output and blood volume are increased in comparison to non-obese subjects¹¹. Concerning liver function, non-alcoholic steatohepatitis is highly associated with morbid obesity patients and histological liver abnormalities such as fatty infiltration are present in approximately 90% of the livers of this population¹²⁻¹³. Furthermore, liver perfusion may be altered due to a combination of ballooning of hepatocytes (due to fatty infiltration) and narrowing of the peripheral hepatic micro vessels, the sinusoids¹⁴. For renal function, it seems that in obese subjects creatinine clearance increases with fat free mass¹⁵ and glomerular filtration is increased in overweight and obese subjects compared to non-obese subjects¹⁶⁻¹⁷.

In addition, it has been reported that obese patients have an increased risk to develop, among others, thrombosis¹⁸, cardiovascular disease¹⁹, cancer²⁰, and infections, including surgical site infections and other nosocomial infections as well as to develop serious complications of common infections²¹. The underlying causes of this increased risk are thought to be related to the metabolic syndrome and the increased inflammation status which are both highly prevalent among subjects with (morbid) obesity²²⁻²⁴.

The physiological changes in obese and morbidly obese patients mentioned above may impact the pharmacokinetics and pharmacodynamics of drugs. Therefore evidence-based dosing guidelines in the (morbidly) obese population are needed, as this population deserves to receive effective and safe pharmacotherapy as much as any other patient group. In particular, in view of the increased risk of obese patients to serious comorbidities, evidence-based dosing is necessary. To reach this goal, knowledge on the extent into which these physiologic changes influence absorption, metabolism, distribution and elimination of drugs is essential. Ultimately also the influence of obesity on the pharmacokinetic-pharmacodynamic relationship should be evaluated.

In the past decades a substantial number of studies have aimed to evaluate how the pharmacokinetics of certain drugs change in obese patients. However, it seems that in the vast majority of these studies only overweight ($\text{BMI} > 25 \text{ kg/m}^2$) and obese ($\text{BMI} > 30 \text{ kg/m}^2$) subjects were included, while today morbidly obese patients ($\text{BMI} > 40 \text{ kg/m}^2$) are not uncommon in the daily practice of physicians and pharmacists. In this respect, there is a strong need for pharmacokinetic studies after both intravenous and oral drug administration in morbidly obese patients to establish evidence-based dosing guidelines for this special population.

BARIATRIC SURGERY

Bariatric surgery or weight loss surgery is considered the most effective treatment option for morbid obesity²⁵⁻²⁶ and results, among other factors, in long term weight loss, reduction in overall mortality and remission of type 2 diabetes²⁶⁻²⁷. Adult patients with a $\text{BMI} > 40 \text{ kg/m}^2$ or with a $\text{BMI} > 35 \text{ kg/m}^2$ with a severe obesity-related co morbid condition such as hypertension, impaired glucose tolerance, diabetes mellitus, hyperlipidemia, or obstructive sleep apnea qualify for a bariatric surgery procedure²⁸⁻²⁹. Bariatric surgery comprises of many different types of surgery of which the Roux and – Y gastric bypass (RYGB, 47% in 2011) and sleeve gastrectomy (SG, 28% in 2011) are currently most performed worldwide, while the adjustable gastric banding procedure has not been performed much in recent years (18% in 2011)³⁰. During a Roux and – Y gastric bypass the stomach is reduced to a small pouch and the duodenum and initial part of the small intestine are bypassed³¹⁻³². For a sleeve gastrectomy a large portion of the stomach along the greater curvature is removed resulting in a tube like structure along the smaller curvature³³. The prevalence of bariatric surgery is increasing, with more than 340,000 bariatric surgeries performed worldwide in 2011³⁰. For the Netherlands, the media reported a significant increase in bariatric surgeries from 4,000 in 2011 to an estimate of 10,000 in 2013³⁴.

Bariatric patients present physicians and pharmacists with many challenges regarding safe and effective drug therapy, as bariatric procedures may impact a drug's pharmacokinetics both due to the anatomical changes made to the gastro-intestinal tract and the induced loss in body weight. These anatomical changes may cause an increase in stomach pH, an increase in gastric emptying time, and a decrease in the surface area of absorption^{31,35}, thus potentially affecting a drug's rate and extent of oral absorption. In addition to these anatomical alterations, bariatric patients may lose a substantial percentage of their body weight (a mean of 32% of total body weight 0.5-2 years after bariatric surgery²⁶) which may have a major impact on the distribution and clearance of a drug³⁶.

While case reports have reported a decrease in oral drug exposure of certain drug in patients after bariatric surgery (e.g. tamoxifen ³⁷, phenytoin ³⁸, imatinib ³⁹), so far only 17 clinical studies have aimed to evaluate how a bariatric procedure alters the pharmacokinetics of approximately 25 drugs ^{31, 36, 40}. The results of this limited number of studies show a large variation. For some drugs, mean oral absorption remains unchanged after a gastric bypass as measured by the oral AUC (e.g. atorvastatin ⁴¹, furosemide and omeprazole ⁴²), while others, including erythromycin ⁴³, tacrolimus and sirolimus ⁴⁴ and tolbutamide ⁴², showed a substantial reduction in AUC after an oral dose. In contrast, for metformin an increase in oral AUC was reported in 16 patients after RYGB ⁴⁵. In summary, there seems to be a large variation in how bariatric surgery influences drug exposure after oral administration ³⁶, whereas it seems that this variation cannot be related to the Biopharmaceutics Classification System (BCS) ⁴⁶. Alternatively, the impact of bariatric surgery on the absorption of a specific drug may be predicted based on the type of surgery and knowledge of the involved absorption processes of the drug (e.g. availability of transporters, bile salts, main oral absorption site, CYP3A substrate, etc.). However, due to the small number of studies, the limited number of patients included, the large variation in compounds studied and types of bariatric surgery included in these studies, no such conclusion can be derived yet.

Finally, information on how loss in body weight in morbidly obese patients after bariatric surgery affects the pharmacokinetics of drugs has only been studied for 8 drugs ^{42, 45, 47-48}, while it is known that increase in body weight greatly affects clearance and particularly distribution of drugs.

THE OBJECTIVE OF THIS THESIS

There is a strong need for knowledge on safe and effective pharmacotherapy in morbidly obese patients and in addition, in patients which have undergone a bariatric procedure.

As a first step towards evidence-based dosing strategies in morbidly obese patients, **Chapter 2** presents an overview of studies on drug pharmacokinetics including both obese and non-obese subjects, sorted by metabolic or elimination pathway of the drug. This overview shows that the impact of obesity on drug metabolism and elimination seems to differ greatly between drugs and depends on the metabolic or elimination pathway primarily involved in the clearance of a drug. In addition, obesity may also impact oral absorption, oral bioavailability and the volume of distribution of drugs. **Chapter 3** provides an overview of the impact of obesity on absorption, distribution, metabolism and elimination of drugs as well as perspectives for future research into the influence of obesity on pharmacokinetics. It shows that (morbid) obesity may largely impact volume of distribution, while the magnitude and direction of change are difficult

to predict, while changes in clearance may be smaller than those on volume of distribution and may be predictable on the basis of the elimination pathway involved. Lastly, it describes that very little is known about the influence of obesity on oral absorption.

The clinical studies presented in this thesis focused on two drugs, i.e. cefazolin and midazolam. In **Chapter 4** a study is presented which aimed to evaluate the influence of morbid obesity on cefazolin pharmacokinetics and penetration into the subcutaneous tissue using clinical microdialysis techniques. Cefazolin is a first generation cephalosporin antibiotic which is widely applied for the prevention of surgical site infections during many types of surgical interventions, including bariatric surgery⁴⁹ and is eliminated completely by glomerular filtration and active tubular excretion⁵⁰. It was studied because in morbidly obese patients there seem to be more surgical wound infections, while cefazolin plasma concentrations seem to reach adequate levels^{21, 51}. It was unknown whether cefazolin reaches adequate levels at the target site, which is the subcutaneous adipose tissue around the surgical wounds, in morbidly obese patients..

Chapter 5 presents a study of midazolam population pharmacokinetics in morbidly obese patients. Midazolam was studied because it is the best Cytochrome P450 3A (CYP3A) substrate currently available and preliminary evidence indicates that absolute clearance of CYP3A substrates is lower in obese patients (Chapter 2)⁵². Midazolam is a widely applied drug for sleeping disorders, (pre)anesthesia and sedation in the Intensive Care. It is metabolized into 1-OH-midazolam by the CYP3A enzyme and is considered a model probe for the activity of CYP3A⁵³⁻⁵⁴. CYP3A is an important enzyme system that is involved in the metabolism of 25% of all clinically used drugs⁵⁵, including many drugs that are relevant for obese patients, such as statins, cardiovascular drugs, antipsychotics and oncolytic drugs⁵⁶. A potentially reduced absolute clearance is highly relevant, because it may result in prolonged effect of the drug with increased risk of adverse events particularly upon prolonged use. As the CYP3A enzyme is located in the liver as well as in the gut wall, it determines both systemic clearance and oral drug bioavailability of CYP3A substrates including midazolam. For this reason a semi-simultaneous dosing design was applied in this study, in which patients first received an oral dose followed by an intravenous dose approximately 150 minutes later. This design allowed for both the evaluation of systemic midazolam clearance as well as midazolam oral bioavailability.

The influence of bariatric surgery on the pharmacokinetics of midazolam was studied in **Chapter 6**. In this study, eighteen patients of the morbidly obese patients who participated in the study of Chapter 5 were restudied one year post bariatric surgery using the same study design. Midazolam systemic clearance and oral bioavailability were closely studied in view of anticipated weight loss together with the anatomical changes to the gastro-intestinal tract, respectively.

To further investigate the CYP3A mediated metabolism of midazolam into 1-OH-midazolam, in **Chapter 7** we used midazolam and 1-OH-midazolam data and semi-phys-

iologically based PK modeling to quantify the influence of bariatric surgery on CYP3A activity in the gut wall and liver separately. This evaluation required a more advanced model in which also presystemic metabolism of midazolam into 1-OH-midazolam could be accounted for.

In **Chapter 8**, the outcomes of the Chapters 4-7 are summarized and interpreted. In addition, perspectives on future studies and directions for evidence-based dosing guidelines in morbidly obese and bariatric patients are provided with the latter being particularly of relevance for clinical practice. Finally, the relevance of these findings for other drugs which share the same elimination pathway are discussed.

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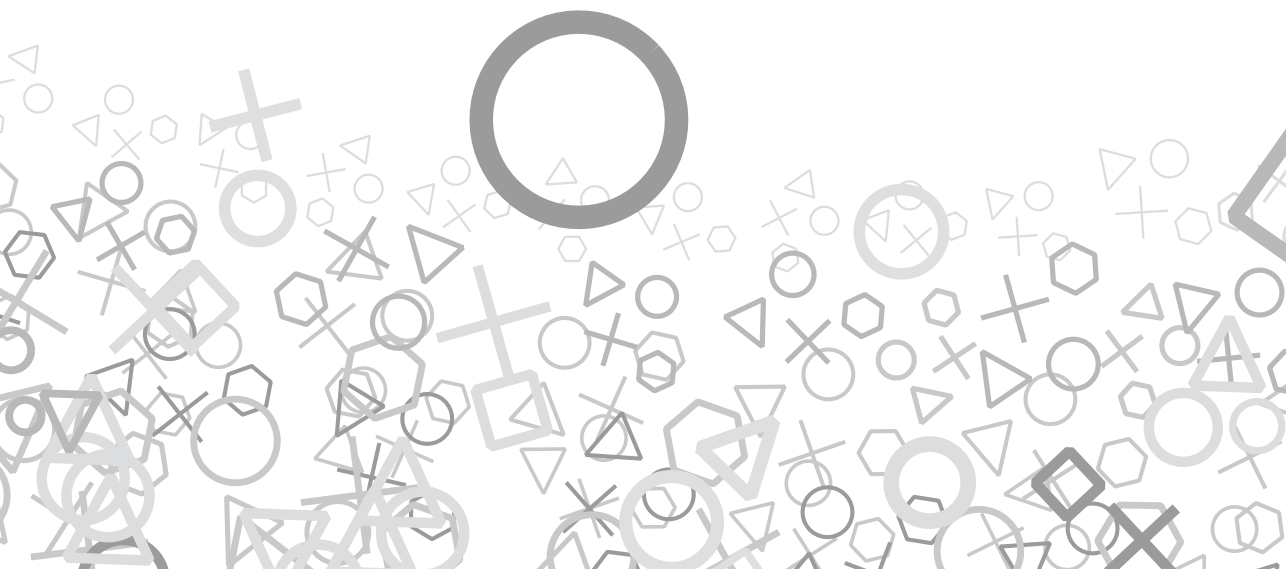


CHAPTER 2

Impact of obesity on drug metabolism and elimination in adults and children

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Clin Pharmacokinet. 2012; 51(5): 277-304



ABSTRACT

The prevalence of obesity in adults and children is rapidly increasing across the world. Several general (patho)physiological alterations associated with obesity have been described, but the specific impact of these alterations on drug metabolism and elimination and its consequences for drug dosing remains largely unknown.

In order to broaden our knowledge of this area, we have reviewed and summarized clinical studies that reported clearance values of drugs in both obese and non-obese patients. Studies were classified according to their most important metabolic or elimination pathway. This resulted in a structured review of the impact of obesity on metabolic and elimination processes, including phase I metabolism, phase II metabolism, liver blood flow, glomerular filtration and tubular processes.

This literature study shows that the influence of obesity on drug metabolism and elimination greatly differs per specific metabolic or elimination pathway. Clearance of cytochrome P450 (CYP) 3A4 substrates is lower in obese as compared with non-obese patients. In contrast, clearance of drugs primarily metabolized by uridine diphosphate glucuronosyltransferase (UGT), glomerular filtration and/or tubular-mediated mechanisms, xanthine oxidase, *N*-acetyltransferase or CYP2E1 appears higher in obese versus non-obese patients. Additionally, in obese patients, trends indicating higher clearance values were seen for drugs metabolized via CYP1A2, CYP2C9, CYP2C19 and CYP2D6, while studies on high-extraction-ratio drugs showed somewhat inconclusive results. Very limited information is available in obese children, which prevents a direct comparison between data obtained in obese children and obese adults.

Future clinical studies, especially in children, adolescents and morbidly obese individuals, are needed to extend our knowledge in this clinically important area of adult and paediatric clinical pharmacology.

INTRODUCTION

Currently more than 30% of the US population is obese (Body Mass Index (BMI) $>30 \text{ kg/m}^2$)¹⁻², while approximately 5% have been reported to be morbidly obese (BMI $>40 \text{ kg/m}^2$)³. In Europe the prevalence of adult obesity ranges from 9-29% depending on the country⁴ and increases every year. Also for children strong upward trends are observed. According to the national health and nutrition examination survey, conducted in 2007 – 2008, 17% of US children are obese⁵. Upcoming economies, such as China and India, also show an alarming increase of obesity in both adults and children with more than 30% of Chinese adults being overweight⁶. If current trends persist, there will be 2.16 billion overweight and 1.12 billion obese individuals worldwide in 2030 as compared with 388–405 million obese individuals in 2005⁷.

In view of this trend, it is important to understand the impact of obesity on drug metabolism and elimination and its consequences for drug dosing in the (morbidly) obese population. Obesity and morbid obesity are associated with several (patho)physiological changes that may influence the pharmacokinetics of drugs. Among other factors, obese patients have relatively more fat and less lean tissue per kilogram of total body weight than non-obese individuals⁸⁻⁹. Blood volume is observed to be increased, particularly in the morbidly obese¹⁰⁻¹¹. In addition, studies have confirmed that obese patients suffer from low-grade inflammation¹², which is probably the underlying cause of the high prevalence of non-alcoholic steatohepatitis (NASH)¹³⁻¹⁴. NASH has been reported to either increase or decrease drug metabolizing enzyme activity¹⁵⁻¹⁸. The net effect of obesity on drug metabolism is also influenced by cardiac output and liver blood flow, both of which are shown to be increased in obese patients¹⁹. Concerning renal function, a state of glomerular hyperfiltration similar to the condition seen in early-stage diabetic nephropathy and sickle cell disease has been reported in obese individuals²⁰⁻²¹. Until now, the influence of obesity on tubular processes has been unknown.

In summary, many (patho)physiological alterations associated with obesity have been described in the literature, yet the impact of these alterations on specific drug metabolic and elimination pathways has not been clearly summarized. Numerous publications have described obesity-related alterations in all aspects of drug pharmacokinetics, including absorption, distribution, metabolism and elimination of drugs^{9, 22-30}. In addition, several publications have tried to provide practical guidelines for dosing in this population^{9, 23-28}. In recent publications, the influence of obesity on drug metabolism and renal elimination was stated to be inconclusive and inconsistent, with drug clearance being the most important pharmacokinetic parameter for maintenance dosing regimens^{9, 22, 24, 27, 30}. In some cases, results from animal or *in vitro* studies have been used to fill the knowledge gaps^{27, 30}. So far, many pharmacokinetic studies have been performed in obese patients and these studies may represent a wealth of knowledge

on clearance of specific drugs in obesity. In this review our goal was to order and sort pharmacokinetic studies by their primary drug metabolic or elimination pathway to gain insight into how these pathways change with obesity. Therefore, drugs representative for a specific pathway were included in the review, in order to generate knowledge on obesity-related changes in the most important metabolic and elimination pathways in humans. As such, this review provides insight into how obesity affects specific drug metabolism and renal elimination pathways in both obese adults and obese children, on the basis of results of pharmacokinetic studies in obese and non-obese individuals. For this purpose, a direct comparison between drug clearance in obese and non-obese individuals is necessary; therefore, clinical trials that included both obese and non-obese individuals were reviewed in this analysis.

SEARCH STRATEGY AND SELECTION CRITERIA

Approach

We studied individual drug metabolism and elimination processes by using drug clearance values as surrogate markers for these processes. To allow for direct comparisons between obese and non-obese individuals, clinical studies that investigated drug pharmacokinetics in both obese and non-obese patients were collected. The drugs reported in these clinical studies were categorized by their currently known rate-limiting clearance processes, and absolute clearance values were summarized in tables, which is an approach that has been applied before²⁹. In addition, weight-normalized clearance values were added to provide information on the weight-normalized changes in clearance values between non-obese and obese individuals. These weight-normalized clearance values were either directly extracted from the original publication or derived by dividing mean clearance by mean total body weight. As an alternative to total body weight, consideration was given to normalizing clearance values for lean body weight, as this parameter is often proposed as a body size descriptor for obese patients^{27, 31}. Unfortunately, this parameter was reported in only very few studies included in this review; therefore, it was not possible to report clearance values adjusted for lean body weight.

Clearance processes were divided into metabolism and renal elimination. For drug metabolism, phase I metabolism, phase II metabolism and liver blood flow were considered. Drugs for which information about the rate-limiting cytochrome P450 (CYP) process was inconclusive were included in the Other Phase I Metabolism section. For renal elimination, two processes involved in drug elimination by the kidneys were identified: glomerular filtration and tubular processes (tubular secretion and tubular reabsorption).

Inclusion criteria

Papers from the international peer reviewed literature reporting drug pharmacokinetics in obese and normal-weight adults or children were eligible for inclusion. Drugs were included if cleared by a specific metabolic or renal elimination pathway, as reported in international peer reviewed literature. This reference about the drug's main metabolic or elimination route was included in the tables.

Search terms and search results

The PubMed database was used for the search for papers in which the pharmacokinetics of a drug were studied in both an obese and non-obese population. The following search terms were used:

- (Clearance[All Fields] AND ('obesity'[MeSH Terms] OR 'obesity'[All Fields])) AND (controls[All Fields] OR normal[All Fields] OR healthy[All Fields]), yielding 562 results on 2 March 2011.
- '[Substrate]' and 'obesity' and 'pharmacokinetics'. Substrates mentioned in Cytochrome P450 Drug Interaction Table were used ³². A total of 91 (CYP3A4), 10 (CYP2E1), 35 (CYP2D6), 43 (CYP1A2), 23 (CYP2C19), 14 (CYP2C9), 1 (CYP2C8), 7 (CYP2B6) papers of interest were found between March and May 2011.
- '[Kidney process]' and 'obesity' and 'pharmacokinetics'. A total of 18 (glomerular), 5 (tubular secretion) and 2 (tubular reabsorption) papers of interest were found between May and June of 2011.

Additionally, references in the selected articles were checked for additional publications to include in this review.

Exclusion criteria

From studies investigating pharmacokinetics of drugs in both obese and non-obese patients, the following studies were excluded: studies on drugs for which the metabolic or renal elimination pathway was reported to be miscellaneous, unknown or inconsistent, as concluded from peer reviewed literature; studies investigating endogenous substances (including insulin); pharmacodynamic studies; animal studies; case reports; and *in vitro* studies.

DRUG METABOLISM

Drug metabolism predominantly occurs in the liver through enzymes responsible for the modification of functional groups (phase I reactions) and the conjugation of endogenous substituents to drugs to make them even more polar (phase II conjugation) ³³.

In 90% of obese patients, histologically proven liver abnormalities as fatty infiltration are present³⁴. Non-alcoholic fatty liver disease (NAFLD) may range from simple liver steatosis without inflammation to non-alcoholic steatohepatitis (NASH) with active hepatic inflammation. NASH prevalence is difficult to assess, because the diagnosis can only be confirmed using a liver biopsy. However, it is estimated that up to 20% of the obese population and up to 50% of morbidly obese patients have NASH³⁵, and its incidence correlates with BMI (kg/m²)³⁶. While fatty infiltration of the liver may result in altered enzyme activity of phase I or II systems, this enzyme activity may also be subject to changes caused by other obesity-associated (patho)physiological changes such as the chronic state of inflammation^{12, 16}.

To describe the enzyme activity of phase I and II systems in obesity, we provide in this section an overview of clinical studies investigating drugs of which clearance is dependent on phase I or II reactions or liver blood flow and which were studied in both obese adults or children and non-obese adults or children in one report.

Phase I metabolism

Phase I enzymes catalyse the modification of functional groups of a substrate (i.e. oxidation, reduction and hydrolysis), and the majority of these enzymes consist of CYPs. CYPs are predominantly located in the endoplasmatic reticulum of hepatocytes. Other sites include the gastro-intestinal tract, where significant amounts of gene expression of various CYP isoforms have been detected³⁷⁻³⁸. CYP enzyme metabolism contributes to approximately 75% of all drug metabolism³⁹. In this section, we provide an updated review of all studies that have investigated phase I-mediated drug clearance in both obese and non-obese patients in one report.

Cytochrome P450 (CYP) 3A4

CYP3A4 is involved in the phase I metabolism of approximately 50% of all drugs⁴⁰. In table I, an overview of the studies comparing clearance of CYP3A4-metabolized drugs in both obese and non-obese individuals is presented. The pharmacokinetics of ten CYP3A4 substrates in obese versus non-obese subjects have been reported, including alfentanil, midazolam, triazolam, alprazolam, cyclosporine, carbamazepine, docetaxel, taranabant, trazodone and *N*-methyl-erythromycin.

As an *in vivo* probe of CYP3A4 activity, *N*-methyl-erythromycin, midazolam, triazolam, alprazolam and cyclosporine are widely applied⁴¹⁻⁴⁶. In this respect, it was reported that obesity was significantly associated with lower metabolism of [¹⁴C]-*N*-methyl-erythromycin, measured as exhaled ¹⁴CO₂ in both men and women ($r^2 = 0.91$ and $r^2 = 0.90$, respectively)⁴¹⁻⁴², indicating reduced CYP3A4 metabolic activity. Similarly, triazolam clearance was significantly lower in obese patients⁴⁷. For midazolam⁴⁸, alprazolam⁴⁷ and cyclosporine⁴⁹⁻⁵⁰, clearance values were reported to be lower in obese versus non-

obese individuals, though this was not statistically significant, potentially because of the limited power of these studies.

A trend towards lower CYP3A4 activity associated with obesity was also found for other major CYP3A4-cleared drugs. Carbamazepine clearance in non-obese versus obese patients was only marginally higher⁵¹. Upon major weight loss, carbamazepine clearance in six obese patients was significantly increased⁵². As an explanation, it has been suggested that a fatty liver, as observed by abdominal ultrasound, may hinder carbamazepine metabolism either by inhibition of important biochemical reactions or by reduction in liver blood flow. After weight loss, ultrasound images showed a disappearance of fatty changes, in line with an increase in carbamazepine clearance. Clearance of alfentanil, which is also predominantly metabolized by CYP3A4⁵³, was almost halved in obese as compared with non-obese patients⁵⁴. The pharmacokinetics of taranabant, primarily metabolized by CYP3A4⁵⁵, were studied using data from 12 phase 1 clinical trials and one phase 2 study, including 385 obese individuals (BMI range 30-43 kg/m²)⁵⁶. While the authors found a lower estimated oral clearance in obese individuals, they attributed this result to either increased protein binding or a decrease in CYP3A activity.

For two CYP3A4 substrates no difference in clearance was reported in obese versus non-obese patients. Trazodone, for which CYP3A4 is the major isoenzyme involved in the formation of its metabolite⁵⁷⁻⁵⁸, showed no difference in clearance between obese and non-obese patients. Furthermore, docetaxel clearance values of adults patients were not significantly different between non-obese, obese or morbidly obese adults⁵⁹.

In studies of patients before and after gastric bypass surgery an increase in activity of CYP3A4 metabolism in obese individuals was reported. Cyclosporine requirement in patients after gastric bypass surgery was significantly increased from 1.8 to 3.5 mg/kg/d ($p=0.02$), in order to maintain similar cyclosporine trough levels⁶⁰. Similarly it was reported that higher, tacrolimus, sirolimus (CYP3A4⁶¹⁻⁶²) and mycophenolic acid (CYP3A4, CYP2C8⁶³) doses were needed in transplant recipients with a gastric bypass to ensure exposure similar to that in a non-bypass patient⁶⁴. In contrast, atorvastatin bioavailability 3 – 6 weeks after gastric bypass surgery was found to be both increased and decreased as compared with before surgery⁶⁵⁻⁶⁶. The observations made in these gastric bypass studies seem to reflect an increase in CYP3A4-mediated clearance in after weight loss. However, these observations may also be explained by the surgical procedures or an increase in activity of CYP3A4 located in the intestines, both causing reduced absorption of oral drugs. Finally, it could be a combination of the factors mentioned. To our knowledge, no studies have investigated the oral bioavailability of CYP3A4 substrates in obese (gastric bypass) patients versus non-obese patients, and as such, we cannot distinguish between these factors.

In summary, 7 out of 13 studies presented in Table 1 show a significantly lower clearance of CYP3A4 substrates in obese patients and 4 studies show non-significantly lower

Table 1 Cytochrome P450 (CYP) 3A4-mediated clearance in both obese and non-obese patients (pts)

Substrate (reference) ^a	Clearance							Reference	
	Obese pts ^b	Non-obese pts ^b	Dose	Parameter	Obese pts ^b	Non-obese pts ^b	Significance		Weight normalized clearance (obese vs non-obese pts) ^{b,c}
Taranabant ⁵⁵	n = 385 BMI 35.4 (3.8) kg/m ²	n = 187 BMI 25 (3.2) kg/m ²	0.5–8.0 mg PO	CL/F	22.4 (CV 44%) L/h	35.6 (CV 51%) L/h	NA	NA	56
Docetaxel ¹⁷⁶	n = 21 BMI >30 kg/m ²	n = 130 BMI <30 kg/m ²	55–100 mg/m ² IV	CL	BMI 30–35 kg/m ² : 46.1 (15) L/h	40.9 (15) L/h	NS	NA	59
Docetaxel ¹⁷⁶	n = 21 BMI >30 kg/m ²	n = 130 BMI <30 kg/m ²	55–100 mg/m ² IV	CL	BMI >35 kg/m ² : 42.3 (13) L/h	40.9 (15) L/h	NS	NA	59
Carbamazepine ⁴⁵	n = 18 TBW 111.4 (20) kg BMI 38.8 (6.0) kg/m ²	n = 13 TBW 63.2 (8.3) kg BMI 22.4 (1.6) kg/m ²	200 mg PO	CL	19.8 (1.2) mL/min	23.0 (1.3) mL/min	p = 0.07	0.18 vs 0.36 mL/min/kg	51
Carbamazepine ⁴⁵	n = 6 TBW 122 (8.4) kg BMI 42.5 (3.2) kg/m ²	n = 6 ^d TBW 92.2 (4.2) kg BMI 32.0 (1.4) kg/m ²	200 mg PO	CL	20.4 (1.8) mL/min	31.6 (5.0) mL/min	p < 0.05	0.17 vs 0.34 mL/min/kg	52
ERBT ⁴⁴	n = 6 TBW >130% IBW	n = 18 Age 70–88 ^e y	0.074 mmol	[¹⁴ C]N-methyl erythromycin	Negative correlation between %IBW and ERBT		p = 0.001	NA	41
ERBT ⁴⁴	n = 5 TBW >130% IBW	n = 4 (hypertensive pts) Age 45–72 ^e y	0.074 mmol	[¹⁴ C]N-methyl erythromycin	Negative correlation between %IBW and ERBT		p < 0.001	NA	42

Table 1 (continued)

Substrate (reference) ^a	Clearance						Reference vs non-obese pts) ^{bc}	Weight normalized clearance (obese pts) ^{bc}
	Obese pts ^b	Non-obese pts ^b	Dose	Parameter	Obese pts ^b	Non-obese pts ^b	Significance	Weight normalized clearance (obese pts) ^{bc}
Midazolam ⁴⁶	n = 20 TBW 116.5 (7.6) kg	n = 20 TBW 65.7 (1.5) kg	5 mg IV, 10 mg PO	CL	472 (38) mL/min	530 (34) mL/min	NS	4.2 vs 8.1 mL/min/kg
Triazolam ⁴³	n = 12 TBW 111.6 (12) kg	n = 12 TBW 63.8 (2.9) kg	1 mg PO	CL	340 (44) mL/min	531 (38) mL/min	p < 0.025	3.05 vs 8.32 mL/min/kg
Alprazolam ⁴³	n = 12 TBW 111.6 (12) kg	n = 12 TBW 63.8 (2.9) kg	0.5 mg PO	CL	66.4 (7.0) mL/min	88 (9.7) mL/min	NS	0.60 vs 1.38 mL/min/kg
Cyclosporine ⁴³	n = 10 TBW 89.7 (11) kg	n = 35 TBW 62.5 (8.4) kg	2.5 mg/kg IV and 14 mg/kg PO (mg/kg)	CL	700 mL/min	780 mL/min	NS	7.80 vs 12.48 mL/min/kg
Cyclosporine ⁴³	n = 13 TBW 102.6 (4.4) kg (>125% IBW)	n = 38 TBW 67.8 (2.2) kg (≤125% IBW)	2.8 (2.0–3.3) mg/kg IV	CL	Lower CL		NS	CL in obese pts is halved when normalized for weight
Trazodone ^{57,58}	n = 23 112 (7) kg	n = 23 TBW 65 (2) kg	25 mg IV, 50 mg PO	CL	146 (10) mL/min	136 (8) mL/min	NS	1.30 vs 2.09 mL/min/kg
Alfentanil ⁵³	n = 6 TBW 123 kg	n = 7 TBW 64 kg	6838 vs 5990 µg	CL	179 mL/min	321 mL/min	p < 0.01	1.46 vs 5.02 mL/min/kg

a The references mentioned with the substrate (first column) refer to literature in which the appropriateness of the particular drug as a CYP3A4 probe was confirmed.
b Unless otherwise specified, mean values (standard deviation or range).

c Body weight normalized clearance values were either taken from the reference or calculated using the mean clearance and mean body weight of the study group. Also see section Approach for calculation of weight-normalized clearance values.
d Same pts after weight loss.

e Values are expressed as range.

BMI = body mass index; CL = drug clearance; CV = coefficient of variation; ERBT = erythromycin breath test; F = bioavailability; %IBW = percentage of ideal body weight; IV = intravenously; NA = not available; NS = not significant; PO = orally; t_{1/2} = elimination half-life; TBW = total body weight.

absolute clearance values. Body weight-normalized clearance values, as depicted in Table 1, show that drug clearance per kilogram body weight is halved in obese individuals. The underlying mechanism of impaired CYP3A4 metabolism and the potential consequences for CYP3A4 drug-drug interactions in obese patients are unclear and should be subjects of future research. Furthermore, it should be noted that the majority of patients included in these studies were mildly obese, while only a few morbidly obese patients (BMI >40 kg/m²) were included. To date, the pharmacokinetics of CYP3A4-metabolized drugs have not been studied in obese children or adolescents.

CYP2E1

Although CYP2E1 metabolism represents only about 5% of phase I drug metabolism³⁹, the impact of obesity on CYP2E1 activity has been the subject of several studies, in which also a significant proportion of morbidly obese patients were included. Chlorzoxazone, enflurane, sevoflurane and halothane represent the four model drugs for CYP2E1 activity reviewed here, of which the results are summarized in Table 2.

Chlorzoxazone pharmacokinetics were studied in several clinical trials, as this drug is a highly selective probe of CYP2E1 metabolism⁶⁷. In women, it was shown that morbid obesity is associated with increased 6-hydroxylation of chlorzoxazone, which is consistent with induction of CYP2E1⁶⁸. For obese patients, with or without non-insulin-dependent diabetes mellitus, it was found that CYP2E1 activity was 40% higher as compared with non-obese subjects^{67,69}.

More recently, CYP2E1 activity in obesity was further studied by Emery *et al.*¹⁸. Unbound oral clearance (CL_{unbound}/F) of chlorzoxazone was approximately 3-fold higher in morbidly obese compared with non-obese individuals (p<0.001). Six weeks and 1 year post-weight-reducing surgery, chlorzoxazone CL_{unbound}/F in patients was reduced. The authors suggest a causal relationship between the induction of CYP2E1 activity and hepatic fatty infiltration, based on liver biopsy assessment. They found a trend towards higher CL_{unbound}/F with increasing severity of liver fatty infiltration or steatosis (P=0.06). More specifically they showed that CL_{unbound}/F was significantly higher among subjects with steatosis involving >50% of hepatocytes, compared with those with steatosis in ≤ 50% of hepatocytes (p=0.02)¹⁸.

Volatile anesthetics, including enflurane, sevoflurane and halothane, are partly metabolized by CYP2E1. Ionic fluoride is formed by CYP2E1 oxidation of enflurane and sevoflurane, and therefore represents a reliable marker of CYP2E1 metabolism⁷⁰⁻⁷¹. A third volatile anesthetic, halothane, undergoes CYP2E1 biotransformation, which results in trifluoro-acetic acid⁷². After a similar dose of enflurane maximal ionic fluoride concentrations were found to be significantly higher in obese compared with non-obese patients⁷³⁻⁷⁴. A similar result was seen for sevoflurane in obese versus non-obese patients⁷⁵. A second sevoflurane study did not find a significant difference in ionic fluoride concentrations between obese

and non-obese patients⁷⁶. After similar doses of halothane, significantly higher trifluoroacetic acid concentrations in obese patients at 1 and 3 hours after dosing were found⁷⁷.

The studies summarized in Table 2 show a consistent and significant increase in clearance of different CYP2E1 substrates in obese as compared with non-obese subjects, indicating induction of CYP2E1 activity in obesity. When normalized for body weight, clearance values are more or less equal among obese and non-obese individuals, which indicates that CYP2E1 activity increases with body weight. As an explanation, liver fatty infiltration, which is expected to increase with increasing body weight, may be the underlying cause of the CYP2E1 enzyme activity increase with body weight¹⁸. In obese children, no studies on CYP2E1-metabolized drugs have been performed yet.

With regard to the higher CYP2E1 activity observed in obese patients, it can be anticipated that caution should be practiced when using paracetamol (acetaminophen) in obese patients, as CYP2E1 catalyses the formation of the toxic metabolite *N*-acetyl-*p*-benzo-quinone imine (NAPQI). Two studies have looked into paracetamol pharmacokinetics in both obese and non-obese patients⁷⁸⁻⁷⁹. Both studies are discussed in the Phase II metabolism section, because 90% of paracetamol is conjugated via phase II metabolism and only 5–10% of paracetamol is metabolized by CYP2E1⁸⁰. Moreover, one study⁷⁹ did not report metabolites, but only paracetamol clearance values, while the other did not measure NAPQI or the metabolites formed after NAPQI (APAP-C or APAP-M)⁷⁸. Therefore, the above-stated warning may be considered somewhat speculative, and further studies are needed to assess the role of CYP2E1 in paracetamol metabolism and toxicity in both obese adults and children – in particular, given the importance of paracetamol in paediatric therapeutics.

CYP2D6

CYP2D6 metabolism represents about 10-15% of phase I drug metabolism in humans⁴⁰. The activity of this CYP isoform may differ greatly between individuals depending on its genetic polymorphisms⁸¹⁻⁸². Two CYP2D6 substrates, dexfenfluramine and nebivolol, have been subjects of pharmacokinetic studies in obese and non-obese individuals, as shown in Table 3.

For dexfenfluramine metabolism, there was a trend towards higher dexfenfluramine clearance and higher metabolite/parent ratio in obese versus non-obese subjects⁸³. Nebivolol clearance was significantly higher in obese subjects as compared with non-obese individuals⁸⁴. As nebivolol clearance is relatively high (>1L/min), it may be more dependent on liver blood flow than on intrinsic CYP metabolism. However, as the CYP2D6 phenotype has been found to influence the clearance of nebivolol, it was included in this section⁸⁵.

In summary, these few studies indicate trends towards increased CYP2D6-mediated metabolism in obese versus non-obese patients.

Table 2 Cytochrome P450 (CYP) 2E1-mediated clearance in both obese and non-obese patients (pts)

Substrate (reference) ^a	Clearance					Reference			
	Obese pts ^b	Non-obese pts ^b	Dose	Parameter	Obese pts ^b		Non-obese pts ^b	Signifi- cance	Weight normalized clearance (obese vs non-obese pts) ^{b,c}
Chlorzoxazone ⁶⁷	n = 16 TBW 172 (104–273) kg BMI 60 (45–100) kg/ m ²	n = 16 TBW 59 (48–93) kg BMI 21 (18–26) kg/ m ²	750 mg PO	CL _{unbound} /F	27.5 (9–55) L/min	9.9 (3–49) L/min	p < 0.001	0.16 vs 0.17 L/min/ kg	18
Chlorzoxazone ⁶⁷	n = 14 TBW 172 (104–273) kg BMI 59 (45–100) kg/ m ²	n = 14 ^d TBW 145 (95–247) kg BMI 50 (41–98) kg/ m ²	750 mg PO	CL _{unbound} /F	26.8 (9–56) L/min	16.6 (8–45) L/min	p < 0.05	0.16 vs 0.11 L/min/ kg	18
Chlorzoxazone ⁶⁷	n = 14 TBW 172 (104–273) kg BMI 59 (45–100) kg/ m ²	n = 14 ^e TBW 118 (61–208) kg BMI 43 (22–82) kg/ m ²	750 mg PO	CL _{unbound} /F	26.8 (9–56) L/min	19.5 (8–50) L/min	NS	0.16 vs 0.17 L/min/ kg	18
Chlorzoxazone ⁶⁷	n = 17 BMI 39 (1.4) kg/m ²	n = 42 TBW within 20% IBW	500 mg PO	Metabolite/ parent drug ratio	0.38 (0.1)	0.30 (0.2)	NS	NA	67, 69
	n = 13 (NIDD pts) BMI 37 (1.5) kg/m ²	n = 42 TBW within 20% IBW	500 mg PO	Metabolite/ parent drug ratio	0.45 (0.2)	0.30 (0.2)	p = 0.007	NA	67, 69
Chlorzoxazone ⁶⁷	n = 9 (women) BMI 35–50 kg/m ² TBW 119 (16) kg	n = 9 (women) BMI 21–30 kg/m ² TBW 72 (11) kg	250 mg PO	CL (parent)				6.2 (1.7) vs 4.2 (0.8) mL/min/kg (p=0.01)	68
				Fractional CL (6-OH metabolite)				4.0 (1.1) vs 2.52 (0.1) mL/min/kg (p=0.006)	68
Enflurane ⁷⁰	n = 26 TBW 128.4 (6.0) kg BMI 46.3 (1.7) kg/m ²	n = 8 TBW 68.1 (1.2) kg BMI 22.9 (2.0) kg/m ² (p > 0.05)	Similar MAC-hr	FL C _{max}	27.8 (2.0) μmol/L	17.0 (3.0) μmol/L	p < 0.01	0.27 vs 0.25 μmol/L/ kg	73

Table 2 (continued)

Substrate (reference) ^a	Clearance					Reference			
	Obese pts ^b	Non-obese pts ^b	Dose	Parameter	Obese pts ^b		Non-obese pts ^b	Signifi- cance	Weight normalized clearance (obese vs non-obese pts) ^{b,c}
Enflurane ⁷⁰	n = 24 TBW 127.6 (6.0) kg BMI 45.9 (1.7) kg/m ²	n = 7 TBW 67.3 (1.2) kg BMI 23.6 (2.0) kg/m ²	Similar MAC-hr	Rate of FL appearance	5.5 μmol/L/h	2.5 μmol/L/h	NA	0.04 vs 0.03 μmol/L/h/kg	73
				Mean FL C _{max}	28.0 (1.9) μmol/L	17.3 (1.3) μmol/L	p < 0.01	0.22 vs 0.26 μmol/L/ kg	74
Sevoflurane ⁷¹	n = 15 TBW 84.8 (2.7) kg BMI 29.3 (0.8) kg/m ²	n = 16 TBW 63.8 (1.5) kg BMI 22.1 (0.4) kg/m ²	Similar MAC-hr (p > 0.05)	All FL concentrations	Higher in obese pts		p < 0.001	NA	75
				Urinary FL excretion	Higher in obese pts (n = 8)		p < 0.001	NA	75
Sevoflurane ⁷¹	n = 13 TBW 114 (8) kg BMI 41 (1) kg/m ²	n = 10 TBW 73 (3) kg BMI 26 (1) kg/m ²	Similar MAC-hr (p > 0.05)	FL C _{max}	49 μmol/L	42 μmol/L	NS	0.43 vs 0.58 μmol/L/ kg	76
Halothane ⁷²	n = 17 TBW 125 (5) kg BMI 45 (1) kg/m ²	n = 8 TBW 59 (5) kg BMI 22 (2) kg/m ²	Similar MAC-hr (p > 0.05)	TFA serum concentration	At T = 1 and 3 h, significantly higher in obese pts		p < 0.05	NA	77

a The references mentioned with the substrate (first column) refer to literature in which the appropriateness of the particular drug as a CYP2E1 probe was confirmed.

b Unless otherwise specified, mean values (standard deviation or range).

c See section Approach for calculation of weight-normalized clearance values.

d 6 wk post-weight-reducing surgery.

e 1 y post-weight-reducing surgery.

f Values are expressed as range.

BMI = body mass index; CL = clearance; CL_{unbound}/F = oral clearance of unbound drug fraction; C_{max} = maximum concentration; FL = ionic fluoride; %BW = percentage of ideal body weight; MAC = minimum alveolar (anaesthetic) concentration; NA = not available; NIDDM = non-insulin-dependent diabetes; NS = not significant; PO = orally; T = time; TBW = total body weight; TFA = trifluoro-acetic acid (metabolite of halothane) ⁷².

Table 3 Cytochrome P450 (CYP) 2D6-mediated clearance in both obese and non-obese patients (pts)

Substrate (reference) ^a	Clearance					Reference
	Obese pts ^b	Non-obese pts ^b	Dose	Obese pts ^b	Non-obese pts ^b	Significance
Dex-fenfluramine ¹⁷⁸	n = 10 BMI 32.2 (2.9) kg/m ²	n = 10 BMI 20.8 (2.0) kg/m ²	30 mg PO	CL 43.9 (21) L/h	CL 37.3 (11) L/h	NS
						Weight normalized clearance (obese vs non-obese pts) ^{b,c}
						NA
Dex-fenfluramine ¹⁷⁸	n = 10 BMI 32.2 (2.9) kg/m ²	n = 10 BMI 20.8 (2.0) kg/m ²	30 mg PO	Parent/ metabolite ratio 2.29 (1.8)	2.05 (1.3)	NS
						NA
Nebivolol ⁸⁵	n = 9 BMI 34.6 (5.6) kg/m ² TBW 99 kg	n = 9 BMI 21.4 (2.6) kg/m ² TBW 60 kg	0.073 mg/kg IBW IV	CL 71.6 (17) L/h	CL 51.6 (11) L/h	p < 0.05
						0.72 vs 0.86 L/h/kg
						84

a The references mentioned with the substrate (first column) refer to literature in which the appropriateness of the particular drug as a CYP2D6 probe was confirmed.
b Unless otherwise specified, mean values (standard deviation).

c See section Approach for calculation of weight-normalized clearance values.

BMI = body mass index; CL = clearance; IBW = ideal body weight; IV = intravenously; NA = not available; NS = not significant; PO = orally; TBW = total body weight.

CYP1A2

CYP1A2 metabolism represents a small part (~5%) of total phase I drug metabolism. Smoking has an inducing effect on CYP1A2 activity⁸⁶. Caffeine and theophylline have been indicated as CYP1A2-specific probes⁸⁷⁻⁸⁸ and have been studied in obese versus non-obese populations by different research groups (Table 4).

In adults, caffeine clearance was not significantly different between non-smoking obese and non-smoking non-obese patients and between obese patients before and after weight loss⁸⁹. Two earlier caffeine studies in adult obese and non-obese subjects also did not show a significant difference in caffeine clearance⁹⁰⁻⁹¹.

In children aged between 6 and 10 years, Chine *et al.* evaluated oxidative enzyme activity of CYP1A2, using the urinary metabolic ratio of caffeine metabolites⁹². The authors observed non-significantly lower CYP1A2 enzyme activity in obese as compared with non-obese children.

Theophylline clearance showed a significant decrease in 16 obese women after a 6.2 (1.5) kg weight loss⁹³. In a study with 200 individuals, no significant difference in theophylline clearance between moderately obese and non-obese subjects was found⁹⁴. However, after correcting for the influence of smoking, higher total body clearance associated with obesity was found for a select group of young non-smoking subjects ($p < 0.025$). In a third study, it was shown that theophylline clearance correlates with total body weight and not with ideal body weight⁹⁵.

In summary, trends of higher clearance values in obese as compared with non-obese patients indicate a slight increase in CYP1A2 activity. When corrected for body weight, clearance values showed both higher and lower clearance values for obese individuals as compared with non-obese subjects (Table 4).

CYP2C9

CYP2C9-mediated metabolism represents about 10% of phase I drug metabolism in humans. For this review, four CYP2C9 substrates (ibuprofen, phenytoin, glimepiride and glipizide) were identified and are presented in Table 5.

Phenytoin and ibuprofen are widely accepted CYP2C9 substrates⁹⁶⁻⁹⁷. Phenytoin and ibuprofen clearance showed a trend towards higher⁹⁸ and significantly⁹⁹ higher clearance in obese patients, respectively. Non-significantly higher CYP2C9 activity in obese subjects was also seen for glimepiride and glipizide. Glimepiride is metabolized primarily by CYP2C9 to the active M1 hydroxy metabolite, the cyclohexyl hydroxymethyl derivative¹⁰⁰. Glimepiride clearance of the parent drug and of the CYP2C9-dependent metabolite M1 were not significantly different in obese versus non-obese type-2 diabetes patients. However, the cumulative urine excretion of M1 over 24 hours post dose was 30% ($p < 0.05$) higher in obese versus non-obese subjects, while both groups received equal doses¹⁰¹. For glipizide (a CYP2C9 substrate¹⁰²), clearance was slightly higher,

Table 4 Cytochrome P450 (CYP) 1A2-mediated clearance in both obese and non-obese patients (pts)

Substrate (reference) ^a	Clearance						Reference
	Obese pts ^b	Non-obese pts ^b	Dose	Parameter	Obese pts ^b	Non-obese pts ^b	Weight normalized clearance (obese vs non-obese pts) ^{b,c}
Caffeine ⁸⁷	n = 9 (children) age 6–10 ^d y TBW >95th %BMI	n = 16 (children) age 6–10 y TBW <84th %BMI	11.5 mg PO	Metabolic ratio	n = 7 5.4 (2.1)	n = 13 6.7 (1.7)	NA 92
Caffeine ⁸⁷	n = 6 TBW 122 (8.4) kg BMI 42.5 (3.2) kg/m ²	n = 6 ^e TBW 92.2 (4.2) kg BMI 32.0 (1.4) kg/m ²	200 mg PO	CL	113.7 (63) mL/min	135.7 (83) mL/min	0.93 vs 1.47 mL/min/kg 89
Caffeine ⁸⁷	n = 14 TBW 110.4 (19) kg BMI 38.5 (5.8) kg/m ²	n = 14 TBW 66.9 (13) kg BMI 22.6 (1.7) kg/m ²	200 mg PO	CL	88.4 (47) mL/min	82.6 (34) mL/min	0.80 vs 1.23 mL/min/kg 89
Caffeine ⁸⁷	n = 3 TBW 110 (27.5) kg	n = 3 TBW 74.0 (7.8) kg BMI 22.6 (1.7) kg/m ²	5.83 mg/kg LBW PO	CL	355 (119) mL/min	219 (45) mL/min	3.23 vs 2.96 mL/min/kg 90
Caffeine ⁸⁷	n = 16 TBW 110 (8) kg	n = 23 TBW 64 (3) kg	162 mg PO	CL	135 (14) mL/min	112 (12) mL/min	1.22 vs 1.75 mL/min/kg 91
Theophylline ⁸⁸	n = 16 (women) TBW 102.8 (21) kg BMI 38.6 (7.8) kg/m ²	n = 16 (women) ^e TBW 6.2 (1.5) kg	250 mg IV	CL	55 (14) mL/min	48 (13) mL/min	0.54 vs 0.50 mL/min/kg 93
Theophylline ⁸⁸	n = 62 TBW 115–155% ^d IBW	n = 133 TBW <115% IBW	Various protocols	CL	62.9 (33) mL/h/kg IBW	55.5 (28) mL/h/kg IBW	NA 94
Theophylline ⁸⁸	n = 5 TBW >155% IBW	n = 133 TBW <115% IBW	Various protocols	CL	59.7 (21) mL/h/kg IBW	55.5 (28) mL/h/kg IBW	NA 94

^a The references mentioned with the substrate (first column) refer to literature in which the appropriateness of the particular drug as a CYP1A2 probe was confirmed.

^b Unless otherwise specified, mean values (standard deviation or range).

^c See section Approach for calculation of weight-normalized clearance values.

^d Values are expressed as range.

^e Same pts after weight loss.

BMI = body mass index; CL = clearance; IBW = ideal body weight; %IBW = percentage of IBW; IV = intravenously; LBW = lean body weight; NA = not available; NS = not significant; PO = orally; TBW = total body weight.

which was not statistically different in obese as compared with non-obese subjects, though the difference in body weight was rather limited¹⁰³.

In summary, these studies indicate slightly increased CYP2C9-mediated clearance in obese as compared with non-obese patients. Body weight-normalized clearance values show a slight decrease in CYP2C9-mediated clearance per kilogram of total body weight (Table 5).

CYP2C19

CYP2C19 biotransformation is involved in approximately 5% of all phase I drug metabolism. As for CYP2D6 and CYP2C9, the activity of this isoform may largely differ depending on genetic polymorphisms¹⁰⁴. Only one clinical study, which is presented in Table 6, investigated the pharmacokinetics of CYP2C19 probes, i.e. diazepam and methyl diazepam¹⁰⁴⁻¹⁰⁵. Diazepam clearance was higher in the obese group, and no difference in desmethyl diazepam clearance in obese versus non-obese individuals was found¹⁰⁶⁻¹⁰⁷. Body weight-normalized clearance values show a slight decrease in CYP2C19-mediated clearance for obese individuals (Table 6).

Other phase I metabolic enzymes

Xanthine oxidase

Besides CYP enzymatic pathways, there is a wide variety of other enzymes contributing to phase I metabolism of drugs. However, often no appropriate substrate for a particular enzyme has been identified¹⁰⁸. We have identified two studies in children, investigating the pharmacokinetics of the xanthine oxidase-metabolized compounds mercaptopurine and caffeine (Table 7).

Mercaptopurine undergoes extensive biotransformation by xanthine oxidase¹⁰⁹. In children, mercaptopurine clearance values were found to be higher in overweight or obese children as compared with non-obese children. In addition, a significant correlation between drug exposure and fat body mass, expressed by the weight/height percentile, was demonstrated¹¹⁰.

Xanthine oxidase also mediates the biotransformation of the caffeine metabolite 1-methylxanthine into 1-methyluric acid, which can be measured in urine. The metabolic ratio for xanthine oxidase, measured using the metabolites in urine, was higher in obese children than in non-obese children between 6 and 10 years of age⁹². Obese children also showed elevated interleukin-6, C-reactive protein, and leptin levels, whereas adiponectin levels were decreased as compared with the non-obese children⁹². It was suggested that these pro-inflammatory cytokines and adipokines upregulate xanthine oxidase gene expression and activity. Another explanation for the increase in xanthine oxidase activity may be the increase in liver volume associated with obesity.

Table 5 Comparison of cytochrome P450 (CYP) 2C9-mediated clearance between obese and non-obese patients (pts).

Substrate (reference) ^a	Clearance						Reference	
	Obese pts ^b	Non-obese pts ^b	Dose	Parameter	Obese pts ^b	Non-obese pts ^b		Signifi- cance
Glimepiride ¹⁰⁰	n=14 T2D, 130 (36) kg	n=14 T2D 72.0 (10) kg	8 mg p.o.	Normalized CL	2.1 (0.1) L/h/1.73 m ²	2.1 (0.8) L/h/1.73 m ²	NS	NA
			8 mg p.o.	Cumulative M1 in 24h urine	130%	100%	P=0.043	NA
Glipizide ¹⁰²	n=12 95.5 (17) kg	n=8 80.8 (10) kg	5 mg p.o.	CL	2.3 (1.0) L/h	2.0 (1.0) L/h	NS	0.024 vs 0.025 L/h/kg
Ibuprofen ¹⁷⁹	n=11 114 (11) kg 38.6 (3.3) kg/m ²	n=11 61 (3) kg 20.7 (0.5) kg/m ²	600 mg p.o.	CL	83 (4) mL/ min	59 (4) mL/ min	P<0.005	0.73 vs 0.97 mL/min/kg
Phenytoin ¹⁸⁰	n=14 124 kg 178% IBW	n=10 67 kg 92% IBW	300 mg i.v.	CLmetabolic	59 (10) mL/ min	39 (3) mL/ min	NS	0.48 vs 0.58 mL/ min/kg

^a The references mentioned with the substrate (first column) refer to literature in which the appropriateness of the particular drug as a CYP2C9 probe was confirmed.
^b Unless otherwise specified, mean values (standard deviation or range).

^c See section Approach for calculation of weight-normalized clearance values.

BMI = body mass index; CL = clearance; %IBW = percentage of ideal body weight; IV = intravenously; M1 = M1 metabolite of glimepiride, cyclohexyl hydroxymethyl derivative; NA = not available; NS = not significant; PO = orally; T2DM = type 2 diabetes mellitus; TBW = total body weight.

Table 6 Cytochrome P450 (CYP) 2C19-mediated clearance in both obese and non-obese patients (pts)

Substrate (reference) ^a	Clearance						Reference		
	Obese pts ^b	Non-obese pts ^b	Dose	Parameter	Obese pts ^b	Non-obese pts ^b		Signifi- cance	Weight normalized clearance (obese vs non-obese pts) ^{b,c}
Diazepam ¹⁰⁵	n=17 101.1 (68-197) kg	n=17 60.4 (49-80) kg	7.5-15 mg i.v.	CL	38.1 (20-80) mL /min	27.3 (20-51) mL /min	P<0.025	0.38 vs 0.45 mL/ min/kg	106
Desmethyl- diazepam ¹⁰⁵	n=12 105 (77-197) kg	n=12 67 (51-91) kg	10.3 mg p.o.	CL	13.2 (7-18) mL /min	13.4 (6-22) mL /min	NS	0.13 vs 0.20 mL/ min/kg	107

a The references mentioned with the substrate (first column) refer to literature in which the appropriateness of the particular drug as a CYP2C19 probe was confirmed.

b Unless otherwise specified, mean values (standard deviation or range).

c See section Approach for calculation of weight-normalized clearance values.

CL = clearance; IV = intravenously; NS = not significant; PO = orally; TBW = total body weight.

Table 7 Other phase I (xanthine oxidase [XO])–mediated clearance in both obese and non-obese patients (pts)

Substrate (reference) ^a	Clearance					Reference		
	Obese pts ^b	Non-obese pts ^b	Dose	Parameter	Obese pts ^b			
Caffeine ⁸⁷	n = 9 (children) age 6–10 ^c y TBW >95th %BMI	n = 16 (children) age 6–10 ^c y TBW <84th %BMI	11.5 mg PO	Metabolic ratio of XO	n = 8 0.7 (0.06)	n = 16 0.6 (0.05) p < 0.001	NA	92
6-mercaptopurine ⁶⁹	n = 9 (children) Age 4–14 ^c y TBW >75th %BMI	n = 9 (children) Age 5–11 ^c y TBW <75th %BMI	Similar doses	CL	206.9 (85) L/h	93.4 (30) L/h p < 0.001	NA	110

a The references mentioned with the substrate (first column) refer to literature in which the appropriateness of the particular drug as an XO probe was confirmed.

b Unless otherwise specified, mean values (standard deviation or range).

c Values are expressed as range.

BMI = body mass index; %BMI = BMI percentile (used in children); CL = clearance; NA = not available; PO = orally; TBW = total body weight; XO = xanthine oxidase.

In conclusion, xanthine oxidase-mediated clearance was significantly increased in obese versus non-obese children in both studies. To our knowledge, no studies on xanthine oxidase in adults have been performed.

Miscellaneous phase I metabolism enzymes

In addition to typical substrates for phase I drug metabolic enzymes, there are many other drugs that undergo hepatic biotransformation by a combination of phase I and phase II enzymes. As a result, even when the exact share of each involved enzyme is known, it is difficult to predict into what extent drug clearance will be affected in obese adults and children. In Table 8 we have summarized all studies in obese and non-obese patients that have investigated the pharmacokinetics of drugs in which multiple enzymes are involved. Here we will only discuss antipyrine, while for the outcomes of other drugs, we refer to Table 8.

Antipyrine (phenazone) is widely used as a model drug in the assessment of hepatic oxidative capacity in humans, as more than 99% of a given dose is excreted into urine as metabolites. The major metabolic routes are *N*-demethylation to norphenazone (CYP2C8, -2C9, -2C18, -1A2), 4-hydroxylation (CYP3A4, -1A2, -2B6) and 3-methylhydroxylation (CYP1A2, -2C9), which together account for 50% – 80% of the dose¹¹¹. Two antipyrine studies reviewed here did not find significantly different clearance values between the obese and non-obese patient groups^{106, 112}.

The outcomes of the antipyrine studies are representative for the general conclusion from the studies in Table 8. In summary, 8 out of 13 studies did not show significantly different clearance values in obese versus non-obese subjects. Of the 5 studies that did find a difference in clearance values, obese clearance values were either higher (doxorubicin, ethinyl-estradiol and bisoprolol¹¹³⁻¹¹⁵) or lower (amiodarone and doxorubicinol¹¹⁶⁻¹¹⁷) as compared with clearance values in the non-obese group. Per kilogram of body weight, all clearance values were lower in obese as compared with non-obese individuals. The limited influence of obesity on these particular clearance values may in part be explained by compensating mechanisms among the different enzymatic pathways involved. However, it should be noted that the differences in body weight between the obese and non-obese subjects in all of the studies in Table 8 are relatively small. As this is a mixed group of drugs, it is difficult to generalize the results.

Summary of phase I metabolism

In summary, phase I enzymatic processes showed higher, lower or similar activity in obese as compared with non-obese subjects, depending on the enzymatic pathway. CYP3A4 mediated clearance was consistently lower, while CYP2E1-mediated clearance showed higher activity among obese versus non-obese adults. For CYP2E1, it has been demonstrated that an increase of CYP2E1-mediated clearance is correlated with both

total body weight and the degree of liver steatosis, supporting the concept that liver fibrosis and inflammation associated with the increase in body weight are the underlying cause of increased CYP2E1 enzyme activity.

Clearance mediated by phase I metabolizing enzymes (CYP1A2, CYP2C9, CYP2C19 and CYP2D6) showed trends of higher clearance values in obese versus non-obese subjects, although in the majority of studies, this was not statistically significant, and the number of studies was limited. In contrast, CYP1A2 activity in children was non-significantly lower in obese versus non-obese children. Xanthine oxidase activity was significantly higher in obese as compared with non-obese children. Overall, the differences in body weight between obese and non-obese individuals were relatively small, and few or no morbidly obese patients were included in these studies.

Phase II metabolism

Phase II metabolic processes include glucuronide-, *N*-acetyl-, methyl-, glutathione- and sulfate- conjugation of substrates. Uridine diphosphate-glucuronosyltransferase (UGT) enzymes catalyze the conjugation of various endogenous substances and exogenous compounds, and are by far the most important phase II processes for metabolism of drugs (~50%)⁴⁰.

Uridine Diphosphate Glucuronosyltransferase (UGT)

The human UGT superfamily is comprised two families (UGT1 and UGT2) and three sub-families (UGT1A, UGT2A, and UGT2B). Many of the individual UGT enzymes are expressed not only in the liver but also in extrahepatic tissues, including the gastrointestinal tract, adipose tissue and kidneys, where the extent of glucuronidation can be substantial¹¹⁸. As the liver is the main UGT enzyme organ, it is suggested that liver disease or increased organ size, often co-occurring with obesity, is somehow correlated with UGT activity. The expression of specific UGT enzymes in visceral and subcutaneous adipose tissue may also provide an explanation for increased UGT activity in obesity¹¹⁹.

Here we will discuss studies of four drugs that primarily undergo UGT conjugation, i.e. paracetamol, garenoxacin, oxazepam and lorazepam. The studies are summarized in Table 9. In contrast to CYP isoforms, individual UGT enzymes responsible for specific drug biotransformation processes were mentioned in an additional column of Table 9.

Paracetamol is extensively metabolized by UGT enzymes¹²⁰⁻¹²¹. In both adult men and women, significantly higher clearance values were found in obese compared with non-obese individuals⁷⁹. Between adolescents with and without NAFLD, no difference in total body weight-normalized clearance was found, indicating higher absolute clearance values in obese adolescents⁷⁸. Furthermore, the ratio of paracetamol/paracetamol-glucuronide metabolite in urine was significantly increased in obese adolescents, indicating increased UGT metabolism.

Table 8 A combination of phase I- and phase II-mediated clearance in both obese and non-obese patients (pts)

Clearance							
	Substrate (reference) ^a	Metabolic enzymes	Obese pts ^b	Non-obese pts ^b	Dose		
Parameter			Obese pts ^b	Non-obese pts ^b		Significance	
Weight normalized clearance (obese vs non-obese pts) ^{b,c}						Reference	
182	Voriconazole ¹⁸¹	CYP2C19, 3A4, 2C9	n = 8 TBW 133 (105–155) kg BMI 46.2 (38–54) kg/m ²	n = 14 TBW 76.9 (7.1) kg BMI 23.7 (1.9) kg/m ²	200 mg CL/F	13.4 (8.5–21) L/h 20.0 (14–26) L/h NS	0.10 vs 0.26 L/h/kg
			n = 8 TBW 133 (105–155) kg BMI 46.2 (38–54) kg/m ²	n = 14 TBW 76.9 (7.1) kg BMI 23.7 (1.9) kg/m ²	300 mg CL/F	10.1 (6.8–44) L/h 8.4 (3.9–13) L/h NS	0.08 vs 0.11 L/h/kg
114	ethynil estradiol ^{183,185}	CYP3A4, 1A2; UGT1A1c, 2C19	n = 15 BMI 33.5 (range 31–36) kg/m ²	n = 13 BMI 22.4 (range 21–24) kg/m ²	30 µg PO AUC ₂₄	1077 (750–1550) pg • h/mL 1414 (1040–1920) pg • h/mL p = 0.04	NA
			C _{min}	C _{min}	31.5 (21–47) pg/mL at 24 h 34.2 (24–50) pg/mL at 24 h NS	NA	

Table 8 (continued)

Substrate (reference) ^a	Metabolic enzymes	Non-obese		Dose	Clearance				Reference	
		Obese pts ^b	Non-obese pts ^b		Parameter	Obese pts ^b	Non-obese pts ^b	Significance		Weight normalized clearance (obese vs non-obese pts) ^{b,c}
Levonorgestrel ^{186,187}	CYP3A4; minor: CYP2E1, 2C19, 2C9	n = 15 BMI 33.5 (range 21–36) kg/m ²	n = 13 BMI 22.4 (range 21–24) kg/m ²	150 µg PO	AUC	85.8 (62–119) ng • mL/mL	79.9 (45–142) ng • mL/mL	NS	NA	114
					C _{min}	2.6 (1.5–4.5) ng/mL at 24 h	2.5 (1.5–4.0) ng/mL at 24 h	NS	NA	
Amiodarone ¹⁸⁸	CYP3A4, 2C8	Total n = 23 BMI (obese pts) 25–31.4 kg/m ²	Total n = 23 BMI (non-obese pts) <25 kg/m ²	2.34 (0.68) mg/kg/d	CL		Decrease of 22.3% when BMI >25	p < 0.005	NA	116
Ifosfamide ¹⁸⁹	CYP3A, 2B	n = 4 TBW 76.8 (70.0–86.0) kg	n = 12 TBW 64.2 (48–77) kg	1.5 g/m ² IV	CL	76.0 (65–92) mL/min	72.2 (53–189) mL/min	NS	0.99 vs 1.14 L/h/kg	190
Antipyrine ^{111, 191}	CYP1A2, 2B6, 2C8, 2C9, 2C18, 3A4	n = 20 TBW 110.4 (19) kg BMI 38.5 (5.8) kg/m ²	n = 11 TBW 62.7 (8.7) kg BMI 22.3 (1.7) kg/m ²	1 g PO	CL	39.3 (12) L/h	34.5 (7.0) L/h	NS	0.36 vs 0.55 L/h/kg	112

Table 8 (continued)

Clearance										
Substrate (reference) ^a	Metabolic enzymes	Obese pts ^b	Non-obese pts ^b	Dose	Parameter	Obese pts ^b	Non-obese pts ^b	Significance	Weight normalized clearance (obese vs non-obese pts) ^{b,c}	Reference
Antipyrine ^{111, 191}	CYP1A2, 2B6, 2C8, 2C9, 2C18, 3A4	n = 6 TBW 122.2 (21) kg BMI 42.5 (7.8) kg/m ²	n = 6 ^e TBW 92.3 (9.1) kg BMI 32.1 (3.0) kg/m ²	1 g PO	CL	41.1 (12) L/min	47.7 (17) L/min	NS	0.34 vs 0.52 L/min/kg	
Antipyrine ^{111, 191}	CYP1A2, 2B6, 2C8, 2C9, 2C18, 3A4	n = 23 TBW 100.3 (59–197) kg	n = 25 TBW 62.5 (49–81) kg	20 mg/kg; maximum 2.0 g IV	CL	38.0 (21–72) mL/min	47.6 (18–103) mL/min	NS	0.38 vs 0.76 mL/min/kg	106
Bisoprolol ¹⁹²	CYP3A4, 2D6	n = 8 (women) TBW 91 (17) kg	n = 8 (women) TBW 51 (4) kg	Similar doses IV	CL	14.8 (1.4) L/h	12.8 (2.2) L/h	p < 0.05	0.163 vs 0.251 L/h/kg	113
Quinine ¹⁹³	CYP3A4, 2D6	n = 9 (Thai pts) TBW 96 (16) kg	n = 8 (Thai pts) TBW 57 (5) kg	600 mg PO	CL	85 (18) L/h	98 (33) L/h	NS	0.89 vs 1.72 L/h/kg	194
Glyburide ¹⁹⁵	CYP3A4, 2C9	n = 12 TBW 100.0 (23) kg BMI 36.0 (9.1) kg/m ²	n = 8 TBW 73.3 (7.2) kg BMI 24.5 (2.0) kg/m ²	20 mg daily dose PO	CL	3.26 (2.2) L/h	3.10 (2.0) L/h	NS	0.03 vs 0.04 L/h/kg	196

Table 8 (continued)

Clearance										
	Parameter	Obese pts ^b	Non-obese pts ^b	Significance	Weight normalized clearance (obese vs non-obese pts) ^{b,c}	Reference				
Substrate (reference) ^a	Metabolic enzymes	Obese pts ^b	Non-obese pts ^b	Dose						
Doxorubicin ¹⁹⁷	Various, including CYP3A4	n = 22 BMI >30 kg/m ²	n = 77 BMI <30 kg/m ²	40–75 mg/m ² IV	CL	BMI 30–35 kg/m ² (15 pts): 65.7 (17) L/h BMI >35 kg/m ² (7 pts): 78.9 (27) L/h	p = 0.045	NA	R115	
Doxorubicin ¹⁹⁷	Various, including CYP3A4	n = 6 (children) age 1–21 ^d y TBW 61.4 kg BMI >30% body fat	n = 16 (children) age 1–21 ^d y TBW 39.6 kg BMI <30% body fat	Based on BSA	Doxorubicin CL	24.6 (2.5) L/h/m ²	26.0 (6.0) L/h/m ²	NS	NA	117
						37.2 (15) L/h/m ²	64.8 (35) L/h/m ²	p = 0.033	NA	117

a The references mentioned with the substrate (first column) refer to literature in which the metabolic enzymes involved in the clearance of the drug are proposed.

b Unless otherwise specified, mean values (standard deviation or range).

c See section Approach for calculation of weight-normalized clearance values.

d Values are expressed as range.

e Same pts after weight loss.

AUC = area under the concentration-time curve; AUC_{0–24} = AUC from 0 to 24 h; BSA = body surface area; CL = clearance; CL/F = oral clearance; C_{min} = minimum concentration; CYP = cytochrome P450; IV = intravenously; M1 = M1 metabolite of glimepiride, cyclohexyl hydroxymethyl derivative; NA = not available; NS = not significant; PO = orally; T2D = type 2 diabetes; UGT = uridine diphosphate glucuronosyltransferase.

Table 9 Uridine diphosphate glucuronosyltransferase (UGT)-mediated clearance in both obese and non-obese patients (pts)

Substrate (reference) ^a	Metabolic enzymes	Clearance								
		Obese pts ^b	Non-obese pts ^b	Dose	Parameter	Obese pts ^b	Non-obese pts ^b	Significance	Weight normalized clearance (obese vs non-obese pts) ^{bc}	Reference
Paracetamol ¹²¹	UGT 1A9, 1A6, 2B15	n = 12 (NAFLD children) age 10–17 ^d y BMI 34.0 (6.1) kg/m ²	n = 12 (children) age 10–17 ^d y BMI 26.2 (11) kg/m ²	5 mg/kg up to 325 mg	Metabolite/parent drug ratio	Increased in obese pts		p = 0.028	NA	78
Paracetamol ¹²¹	UGT 1A9, 1A6, 2B15	n = 12 (NAFLD children) BMI 34.0 (6.1) kg/m ²	n = 12 (children) BMI 26.2 (11) kg/m ²	5 mg/kg up to 325 mg	CL				0.31 vs 0.31 L/kg/h (NS)	
Paracetamol ¹²¹	UGT 1A9, 1A6, 2B15	n = 7 (men) TBW 135 kg	n = 10 (men) TBW 71 kg	650 mg IV over 5 min	CL	484 mL/min	323 mL/min	p < 0.05	3.59 vs 4.55 mL/min/kg	79
Paracetamol ¹²¹	UGT 1A9, 1A6, 2B15	n = 14 (women) TBW 88 kg	n = 11 (women) TBW 55 kg	650 mg IV over 5 min	CL	312 mL/min	227 mL/min	p < 0.05	3.55 vs 4.13 mL/min/kg	
Garenoxacin ¹²²	Sulfate conjugation of UGT	n = 196 TBW >130% IBW	n = 384 TBW <130% IBW	Various protocols	CL	Obesity (>130% IBW) was a covariate on CL		p < 0.00001	NA	123
Oxazepam ¹²⁰	UGT 1A9, 2B7, 2B15	n = 11 TBW 115 (13) kg	n = 11 TBW 60 (2.6) kg	30 mg PO	CL	156.8 (23) mL/min	50.4 (6.0) mL/min	p < 0.001	1.39 vs 0.82 mL/min/kg (p < 0.005)	124
Lorazepam ¹²⁵	Various, including UGT 2B15	n = 14 TBW 111.7 (10) kg	n = 14 TBW 62.8 (2.2) kg	2 mg IV	CL	102 (10) mL/min	62.9 (5.4) mL/min	p < 0.005	0.98 vs 1.00 mL/min/kg	124

^a The references mentioned with the substrate (first column) refer to literature in which the appropriateness of the particular drug as an UGT probe was confirmed.

^b Unless otherwise specified, mean values (standard deviation).

^c See section Approach for calculation of weight-normalized clearance values.

^d Values are expressed as range.

BMI = body mass index; BSA = body surface area; CL = clearance; C_{min} = minimum concentration; %IBW = percentage of ideal body weight; IV = intravenously; NA = not available; NAFLD = non-alcoholic fatty liver disease; NS = not significant; PO = orally; TBW = total body weight.

In a population pharmacokinetic analysis of garenoxacin (a major UGT substrate ¹²²), it was found that clearance values increased with total body weight. In the final pharmacokinetic model, an obesity factor (>130% ideal body weight) used as a covariate for clearance significantly improved the model ¹²³.

For both oxazepam and lorazepam, it was found that clearance values were significantly higher in obese as compared with non-obese control subjects ¹²⁴. A determinant role of UGT in the metabolism of both compounds has been shown in the literature ^{120, 125}. On the basis of the differences in oxazepam and lorazepam clearance values, the authors concluded that obesity is associated with an increased conjugating capacity and that this increase is in proportion to total body weight. It should be noted that many subjects in this study received more than one study drug, which may limit the interpretation of these results.

In conclusion, all studies show a significantly increased clearance in obese as compared with non-obese subjects. As a consequence, body weight-normalized clearance values were equal or only slightly lower for obese as compared with non-obese individuals, except for oxazepam clearance, which showed a significant increase in body weight-normalized clearance.

Other metabolic phase II routes

Apart from UGT, the pharmacokinetics of *N*-acetyltransferase (~5% of phase II drug metabolism) and glutathione *S*-transferase-metabolized drugs have been investigated in obese versus non-obese subjects. Caffeine, procainamide and busulfan have been indicated as substrates, as presented in Table 10.

N-acetyltransferase is responsible for the *N*-acetylation of procainamide ¹²⁶. Procainamide plasma clearance was slightly higher in obese as compared with non-obese adults, although this was non-significant ¹²⁷. In obese children, a 5-fold increase in the metabolic ratio of the *N*-acetyltransferase pathway of caffeine was observed when compared with non-obese children ⁹², when only considering the slow-acetylator genotype.

For busulfan, both obese (BMI between 27 and 35 kg/m²) and severely obese patients (BMI > 35 kg/m²) showed significantly higher oral clearance values as compared with non-obese patients ¹²⁸. Per kilogram of body weight, clearance was significantly lower in obese versus non-obese patients. This was confirmed in a more recent trial with busulfan in obese and non-obese adults ¹²⁹. While CYP3A4 involvement is suggested ¹³⁰, the glutathione *S*-transferase A1-1 isoform is the major and possibly determinant pathway of busulfan metabolism ¹³¹. In obese children (aged 0 – 21 years) busulfan clearance per kilogram of body weight after a test dose and a regular dose was lower than in non-obese children ¹³².

In conclusion, other type phase II-metabolized substrates show higher absolute clearance values in obese as compared with non-obese adults and children, while weight-normalized clearance values were lower in obese as compared with non-obese patients.

Table 10 Other phase II-mediated clearance in both obese and non-obese patients (pts)

Clearance										
Substrate (reference) ^a	Metabolic enzymes	Obese pts ^b	Non-obese pts ^b	Dose	Parameter	Obese pts ^b	Non-obese pts ^b	Significance	Weight normalized clearance (obese vs non-obese pts) ^{b,c}	Reference
Caffeine ⁸⁷	NAT2	n = 9 (children) age 6–10 ^c y TBW >95% BMI	n = 16 (children) age 6–10 ^c y TBW <84% BMI	11.5 mg PO	Metabolic ratio of NAT2	n = 6 1.01 (0.3)	n = 14 0.18 (0.1)	p < 0.01	NA	92
Procainamide ^{92,126,178}	NAT2	n = 7 TBW 100.2 (17.3) kg	n = 7 TBW 68.4 (11.5) kg	300 mg IV	CL _{plasma}	51.7 (9.2) L/h	41.9 (14) L/h	p = 0.085	0.52 vs 0.61 L/h/kg	127
Busulfan ¹³¹	GSTA1	n = 22 (children) age 0–21 ^d y TBW >85% BMI	n = 29 (children) age 0–21 ^d y TBW 25–85% BMI	Test dose 0.8 mg/kg IV	CL				3.2 vs 3.8 mL/min/kg (p = 0.1)	132
Busulfan ¹³¹	GSTA1	n = 22 (children) age 0–21 ^d y TBW >85% BMI	n = 29 (children) age 0–21 ^d y TBW 25–85% BMI	2.9 vs 4.0 mg/kg IV	CL				3.1 vs 3.8 mL/min/kg (p = 0.03)	132
Busulfan ¹³¹	GSTA1	n = 39 BMI 27–35 kg/m ²	n = 71 BMI 18–26.9 ^d kg/m ²	0.8 mg/kg IBW, TBW or AIBW IV	CL				2.33 vs 2.63 mL/min/kg (p < 0.05)	129
Busulfan ¹³¹	GSTA1	n = 11 BMI >35 kg/m ²	n = 71 BMI 18–26.9 ^d kg/m ²	0.8 mg/kg IBW, TBW or AIBW IV	CL				1.88 vs 2.63 mL/min/kg (p < 0.05)	129
Busulfan ¹³¹	GSTA1	n = 89 BMI 27–35 ^d kg/m ²	n = 173 BMI 18–27 ^d kg/m ²	0.4–1.8 mg/kg PO	CL/F	223 (53) mL/min	190 (45) mL/min	p < 0.001	2.56 vs 2.90 mL/min/kg (p < 0.01)	128
Busulfan ¹³¹	GSTA1	n = 10 BMI >35 kg/m ²	n = 173 BMI 18–27 ^d kg/m ²	0.4–1.8 mg/kg PO	CL/F	250 (47) mL/min	190 (45) mL/min	p = 0.001	2.30 vs 2.90 mL/min/kg (p < 0.01)	128

a The references mentioned with the substrate (first column) refer to literature in which the appropriateness of the particular drug as a NAT2 or GSTA1 probe was confirmed.

b Unless otherwise specified, mean values (standard deviation).

c See section Approach for calculation of weight-normalized clearance values.

d Values are expressed as range.

AIBW = adjusted ideal body weight; BMI = body mass index; %BMI = BMI percentile (used in children); CL = clearance; CL/F = oral clearance; GSTA1 = glutathione S-transferase A1; IBW = ideal body weight; IV = intravenously; NA = not available; NAFLD = non-alcoholic liver fatty disease; NAT2 = arylamine *N*-acetyltransferase type 2; PO = orally; TBW = total body weight.

Summary of phase II metabolism

For glucuronidation processes, all studies in Table 9 show a significant increase in UGT biotransformation in obese as compared with non-obese subjects. Weight-normalized UGT clearance values were equal to or only slightly lower in obese as compared with non-obese patients. However, the number of studies with UGT-metabolized drugs is small. The underlying mechanism of this phenomenon remains unsolved, although NAFLD was demonstrated to be associated with higher paracetamol clearance values in adolescents ⁷⁸.

N-Acetylation catalyzed by *N*-acetyltransferase shows a significant increase in obese children and a non-significant increase in adults. Glutathione transferase of busulfan in obese children and adults was lower in non-obese adults and children when normalized for body weight.

Liver blood flow

High-extraction-ratio drugs are rapidly metabolized and therefore sensitive to changes in liver blood flow, but are relatively insensitive to changes in enzyme activity and are thus a potential marker of liver blood flow. The influence of obesity on liver blood flow is not fully specified. NASH increases fat deposition in the liver, causing sinusoidal narrowing and altered functional morphology of the liver ¹³³. In contrast, because of increased blood volume and cardiac output, liver blood flow is not necessarily reduced in obese subjects ¹⁹.

In Table 11, studies of eight high extraction ratio drugs in obese and non-obese subjects are summarized and include propofol, propranolol, labetalol, verapamil, lidocaine, fentanyl, sufentanil and paclitaxel.

Propofol is extensively metabolized by various UGT enzymes ¹¹⁸ and its clearance is limited by liver blood flow ¹³⁴. Van Kralingen *et al.* ¹³⁵ and Cortinez *et al.* ¹³⁶ studied propofol pharmacokinetics in a wide range of body weights and found that total body weight as a covariate for clearance significantly improved the predictive performance of the population pharmacokinetic model.

Four different studies reported propranolol clearance values in obese versus non-obese patients. Three studies did not show altered clearance values between obese and

Table 11 Liver blood flow-mediated clearance in both obese and non-obese patients (pts)

Substrate (reference) ^a	Clearance					Weight normalized clearance (obese vs non-obese pts) ^{b,c}	Refer- ence		
	Parameter	Obese pts ^b	Non-obese pts ^b	Dose	Obese pts ^b				
Propofol ¹³⁴	n = 20 BMI 43 (6) kg/m ² TBW 124 (20) kg	n = 44 BMI 25 (4) kg/m ² TBW 74 (11) kg	Continuous infusion	CL	Individual clearance (L/min)= 2.22 • (70/ TBW) ^{0.71}	At 70 kg: 2.22 L/min	p < 0.005	NA	135
Propofol ¹³⁴	n = 27 TBW 82–169 ^d kg	n = 24 TBW 44–122 ^d kg	Continuous infusion	CL	Individual clearance (L/min)= 2.25 • (70/ TBW) ^{0.75}	At 70 kg: 2.25 L/min	p < 0.01	NA	136
Paclitaxel ¹⁹⁸	n = 14 BMI >30 kg/m ²	n = 38 BMI <30 kg/m ²	50–225 mg/ m ²	CL	383 (340–431) L/h	318 (291–347) L/h	NS	NA	59
Sufentanil ¹⁴⁵⁻¹⁴⁶	n = 8 TBW 94.1 (14) kg	n = 8 TBW 70.1 (13) kg	4 µg/kg TBW IV	CL	1.99 (0.9) L/min	1.78 (0.5) L/min	NS	0.02 vs 0.03 L/min/kg	148
Propranolol ¹⁹⁹	n = 9 (normolipidaemic pts) BMI 35.6 (1.2) kg/m ²	n = 18 BMI 24.0 (0.6) kg/ m ²	80 mg PO	CL/F	66.2 (22) L/h	73.1 (16) L/h	NS	NA	137
Propranolol ¹⁹⁹	n = 16 (hyperlipidaemic pts) BMI 35.6 (1.5) kg/m ²	n = 18 BMI 24.0 (0.6) kg/ m ²	80 mg PO	CL/F	63.4 (21) L/h	73.1 (16) L/h	NS	NA	137
Propranolol ¹⁹⁹	n = 9 BMI 34.6 (5.6) kg/m ² TBW 99 kg	n = 9 BMI 21.4 (2.6) kg/ m ² TBW 60 kg	0.108 mg/kg IBW	CL	46.2 (10.5) L/h	41.6 (6.8) L/h	NS	0.47 vs 0.69 L/h/kg	138
Propranolol ¹⁹⁹	n = 12 TBW 110.3 (20.4) kg	n = 12 TBW 66.7 (6.8) kg	8 mg IV	CL	57.5 (18) L/h	75.9 (15) L/h	p < 0.01	0.52 vs 1.14 L/h/kg	140
Propranolol ¹⁹⁹	n = 6 TBW 136.5 (36) kg	n = 6 TBW 66.8 (11) kg	10 mg IV	CL	780 (20) mL/min	780 (10) mL/min	NS	5.73 vs 11.67 mL/ min/kg	139

Table 11 (continued)

Substrate (reference) ^a	Obese pts ^b	Non-obese pts ^b	Dose	Clearance			Signifi- cance	Weight normalized clearance (obese vs non-obese pts) ^{b,c}	Refer- ence
				Parameter	Obese pts ^b	Non-obese pts ^b			
Propranolol ¹⁹⁹	n = 6 TBW 136.5 (36) kg	n = 6 TBW 66.8 (11) kg	40 mg PO	CL	2.4 (0.2) L/min	2.8 (0.2) L/min	NS	0.02 vs 0.04 L/min/kg	139
Labetalol ²⁰⁰	n = 9 TBW 99 (23) kg BMI 34.6 (5.6) kg/m ²	n = 9 TBW 60 (11) kg BMI 21.4 (2.6) kg/ m ²	Mean 60.7–61.9 mg (p > 0.05)	CL	89.9 (11) L/h	81.5 (15) L/h	NS	0.91 vs 1.36 L/h/kg	138
Verapamil ²⁰¹⁻²⁰²	n = 12 (hypertensive pts) TBW 127 (8) kg	n = 11 (hypertensive pts) TBW 74 (4) kg	0.15 mg/kg; maximum 25 mg IV	CL	1.34 (0.2) L/min	1.25 (0.1) L/min	NS	0.01 vs 0.02 L/h/kg	142
Lidocaine ²⁰³	n = 14 (men) TBW 124 (8) kg	n = 19 (men) TBW 69 (1) kg	25 mg IV	CL	1427 (120) mL/min	1346 (86) mL/ min	NS	11.51 vs 19.51 mL/ min/kg	143
Lidocaine ²⁰³	n = 11 (women) TBW 96 (6) kg	n = 12 (women) TBW 59 (2) kg	25 mg IV	CL	1089 (83) mL/min	1162 (84) mL/ min	NS	11.34 vs 19.69 mL/ min/kg	143
Fentanyl ^{145,204}	n = 10 TBW 117.3 (33) kg	n = 16 TBW 68.5 (9.5) kg	Bolus and infusion rate based on kg TBW	CL	986 (155) mL/min	718 (163) mL/ min	p < 0.001	8.76 vs 10.48 mL/ min/kg (p < 0.025)	149

^a The references mentioned with the substrate (first column) refer to literature in which the drug is mentioned as a high-extraction-ratio drug.

^b Unless otherwise specified, mean values (standard deviation or range).

^c See section Approach for calculation of weight-normalized clearance values.

^d Values are expressed as range.

BMI = body mass index; CL = clearance; CL/F = oral clearance; IBW = ideal body weight; IV = intravenously; NA = not available; NS = not significant; PO = orally; TBW = total body weight.

non-obese patients¹³⁷⁻¹³⁹, and one study found significantly lower propranolol clearance values in obese versus non-obese patients¹⁴⁰. Propranolol clearance is strongly determined by liver blood flow as it approaches liver blood flow values¹⁴¹. On the other hand, propranolol tends to decrease liver blood flow by ~20-30% by blocking the beta-adrenoreceptor, explaining the relative lower clearance value seen for propranolol compared with other drugs in Table 11¹⁴¹.

Labetalol clearance in obese patients showed a trend towards being increased¹³⁸. For verapamil and lidocaine, no difference in clearance between obese and non-obese was found¹⁴²⁻¹⁴³. As lidocaine clearance is determined mainly by liver blood flow¹⁴⁴, the authors concluded that extreme total body weights did not change liver blood flow.

Sufentanil and fentanyl are predominantly metabolized by CYP3A4¹⁴⁵, but their total clearance is mainly determined by liver blood flow¹⁴⁶⁻¹⁴⁷. Sufentanil showed higher clearance values in obese versus non-obese patients; however, this difference was not statistically significant¹⁴⁸. The difference in body weight between the two groups studied was small (90 versus 74 kg). The pharmacokinetics of fentanyl were studied in a population with a wide range of total body weights, showing a non-linear positive correlation between total body weight and fentanyl clearance¹⁴⁹. Reported paclitaxel clearance values in obese and non-obese patients are extremely high (291 – 431 L/h), indicating liver blood flow-dependent clearance¹⁵⁰. Clearance values for paclitaxel in obese patients were higher than values of non-obese patients; however, this was not statistically significant⁵⁹.

In conclusion, only a few high-extraction-ratio drug studies in Table 11 showed altered clearance values in obese versus non-obese adults. Body weight-normalized clearance values show a large decrease in clearance per kilogram. For instance, the clearance per kilogram values of propranolol and lidocaine are almost halved. A straightforward conclusion from these studies is complicated because of the heterogeneity of the drugs. Liver blood flow is about 2–2.5 L/min, while clearance values of some drugs listed in table XI are less than 1 L/min, obscuring the justification of their role as a model drug for liver blood flow. When considering drugs with clearance values of more than 1.5 L/min (propofol, sufentanil and paclitaxel), all studies show higher clearances in obese patients. Propranolol was excluded from this comparison, as this drug shows high variability in drug clearance values among studies (Table 11). The observation of increased clearance is not statistically significant for sufentanil and paclitaxel, probably because of the small difference in total body weight in these studies. Unfortunately, the data from these studies did not allow comparison of weight-normalized clearance values.

Summary of liver blood flow

According to the results of propofol, sufentanil and paclitaxel studies, liver blood flow is likely to be increased in obese patients. However, only a few (very) high-extraction-ratio drugs have been studied and the difference in body weights between patients groups

was limited for sufentanil and paclitaxel. To our knowledge, no studies have investigated the pharmacokinetics of high-extraction-ratio drugs in children.

RENAL ELIMINATION

The kidneys are the primary organs involved in the elimination of drugs. The processes involved in drug elimination through the kidneys include glomerular filtration, tubular secretion and tubular reabsorption. The exact effect of obesity on these functions is not clear²⁵. Renal function seems to be affected, as obese patients showed a 62% increase in the mean estimated glomerular filtration rate (eGFR)¹⁵¹. This finding was observed, irrespective of the presence of hypertension by an increase of renal blood flow¹⁵². Obesity is related to a state of glomerular hyperfiltration, which resembles that seen in early-stage diabetic nephropathy and sickle cell disease^{20-21, 153}. It has been argued that overweight may ultimately lead to end-stage renal disease, because focal glomerular sclerosis and/or diabetic nephropathy have been observed in a small study in 17 morbidly obese patients who presented with proteinuria¹⁵⁴. In obese children, it was found that the glomerular filtration rate increases with BMI¹⁵⁵. In contrast to obese adults, obese children showed a higher degree of albuminuria, a marker for glomerular dysfunction¹⁵⁶⁻¹⁵⁷. Therefore, it was concluded that albuminuria indicates early renal glomerular dysfunction as a consequence of childhood obesity¹⁵⁶. However, obese children compared with non-obese children did not differ in their glomerular filtration rates, as no overt changes in eGFR were detected¹⁵⁷.

The influence of obesity on renal tubular secretion and renal tubular reabsorption is not well known, and no objective clinical measure of these drug clearance pathways presently exists¹⁵¹. Tubular dysfunction can be defined as the presence of at least two of the following criteria: nondiabetic glycosuria, urine phosphate wasting, hyperaminoaciduria, beta-2-microglobulinuria, and increased fractional excretion of uric acid¹⁵⁸⁻¹⁵⁹. For obese children, an increased degree of beta-2-microglobulinuria, suggesting increased tubular dysfunction, has been described¹⁵⁶.

In this section, we will provide an overview of clinical studies investigating drugs that are primarily eliminated renally and were studied in both non-obese and obese adults and children.

Glomerular filtration

In Table 12, an overview of studies comparing clearance of drugs that are mainly excreted by glomerular filtration in obese and non-obese individuals is presented. These drugs include vancomycin, daptomycin, carboplatin, low-molecular-weight heparins and cimetidine.

Table 12 Glomerular filtration-mediated clearance in both obese and non-obese patients (pts)

Substrate (reference) ^a	Clearance						Weight normalized clearance (obese vs non-obese pts) ^{b,c}	Refer- ence	
	Obese pts ^b	Non-obese pts ^b	Dose	Parameter	Obese pts ^b	Non-obese pts ^b			Signifi- cance
Vancomycin ²⁰⁵	n = 24 TBW 165 (46) kg	n = 24 TBW 68 (6) kg	TDM guided	CL	197 (77) mL/ min	77 (22) mL/ min	p < 0.001	1.19 vs 1.13 mL/ min/kg	160
Daptomycin ²⁰⁶	n = 7 TBW 114 (16) kg	n = 7 TBW 59 (6) kg	4 mg/kg based on TBW	CL	0.82 (0.21) L/h	0.73 (0.14) L/h	p = 0.34	0.43 vs 0.74 L/min/kg	161
Daptomycin ²⁰⁶	n = 7 (obese pts) TBW 86 (9) kg	n = 12 TBW 64 (7) kg	4 mg/kg based on TBW	CL	0.86 (CV8%) L/h	0.72 (CV 6%) L/h	p = 0.008	10.07 vs 11.89 mL/h/ kg	162
	n = 6 (morbidly obese pts) TBW 126 (17) kg				1.01 (CV 29%) L/h	0.70 (CV 34%) L/h	p = 0.003	7.82 vs 10.19 mL/h/ kg	
Carboplatin ¹⁶³	n = 15 BMI >30 kg/m ²	n = 218 BMI <30 kg/m ²	Protocol based	CL	Related to adjusted IBW		No dose adjustment needed in obese pts	NA	165
Carboplatin ¹⁶³	n = 14 BMI >30 kg/m ²	n = 64 BMI <30 kg/m ²	Based on renal function	CL	6.48 L/h	5.88 L/h	p = 0.37	NA	(59)
Carboplatin ¹⁶³	n = 43 BMI >30 kg/m ²	n = 285 BMI >18.5 kg/m ² and <30 kg/m ²	Based on renal function	CL	Increased with TBW		No dose adjustment needed in obese pts	NA	164
Enoxaparin ²⁰⁷	n = 118 TBW 43–120 ^d kg		1.0–1.5 mg/kg bid	CL	CL = 0.3 • CL _{cr} /70 + 0.42 • LBW/55 (kg)		p < 0.001	NA	208
Dalteparin ²⁰⁷	n = 10 TBW 106 (22) kg	n = 10 TBW 70 (9) kg	Protocol based	CL	1.30 L/h	1.11 L/h	NS	0.74 vs 0.95 L/min/kg	209

Table 12 (continued)

Substrate (reference) ^a	Obese pts ^b	Non-obese pts ^b	Dose	Clearance				Reference	
				Parameter	Obese pts ^b	Non-obese pts ^b	Signifi- cance		Weight normalized clearance (obese vs non-obese pts) ^{b,c}
Tinzaparin ²⁰⁷	n = 425 TBW 37–151 ^d kg		175 IU/kg od	CL	22% decrease in CL in L/h/ kg in obese pts (BMI > 30 kg/m ²)			NA	210
Cimetidine ²¹¹	n = 13 TBW 113 (9) kg	n = 16 TBW 64 (2) kg	200–300 mg IV	CL	616 (34) mL/ min	579 (39) mL/min	NS	5.45 vs 9.05 mL/ min/kg	167

a The references mentioned with the substrate (first column) refer to literature in which the appropriateness of the particular drug as a GFR probe was confirmed.
b Unless otherwise specified, mean values (standard deviation).

c See section Approach for calculation of weight-normalized clearance values.

d Values are expressed as range.

bid = twice daily; BMI = body mass index; CL = clearance; CL_{Cr} = creatinine clearance; CV% = coefficient of variation; GFR = glomerular filtration rate; IBW = ideal body weight; IV = intravenously; LBW = lean body weight; NA = not available; NS = not significant; od = once daily; TBW = total body weight; TDM = therapeutic drug monitoring.

Vancomycin clearance in morbidly obese patients is reported to increase with total body weight, compared with non-obese patients¹⁶⁰. No significant increase of daptomycin clearance was described in obese patients with a mean total body weight of 114 kg¹⁶¹. However, in patients with a higher mean total body weight (126 kg), significantly higher daptomycin clearance was reported¹⁶². Carboplatin is mainly eliminated by glomerular filtration and partly by tubular secretion¹⁶³. Both a linear increase of carboplatin clearance with total body weight¹⁶⁴ and ideal body weight¹⁶⁵ have been described. A comparison of carboplatin clearance values between obese and non-obese patients showed no significant difference⁵⁹. The low-molecular-weight heparins enoxaparin, tinzaparin and dalteparin show higher total drug clearance in obese patients compared with non-obese patients (166-168). Studies on the influence of obesity on drug clearance mediated by glomerular filtration in obese children are very limited. In obese children, lower anti-Xa levels after the same dose of enoxaparin were reported, suggesting higher enoxaparin clearance in obese children¹⁶⁶. In contrast to these studies, total clearance of cimetidine was not altered in obese patients compared with non-obese patients¹⁶⁷.

In conclusion, the majority of these studies show higher clearance values with increasing body weights, indicating increased glomerular filtration in obese patients. Weight-normalized clearance values did not show a consistent trend for the influence of overweight on glomerular filtration, as normalized clearance values were either equal or lower in obese as compared with normal-weight patients.

Tubular secretion

Drugs that are (partly) eliminated by tubular secretion and have been investigated in obese patients are summarized in Table 13 and include procainamide, ciprofloxacin, cisplatin, topotecan and digoxin.

Approximately 50% of administered procainamide is eliminated as unchanged drug by glomerular filtration and active tubular secretion¹⁶⁸. Renal procainamide clearance was shown to be higher in obese patients because of elevated tubular secretion, as no significant difference in 24-hour creatinine clearance was observed between obese and non-obese patients¹²⁷. Significantly higher clearance values were also reported for cisplatin and ciprofloxacin, which are eliminated by tubular secretion^{59, 169-171}. For both topotecan and digoxin, there was a trend towards higher drug clearance in obese patients, which is assumed to result from increased tubular secretion^{59, 172}. For tubular secretion, normalized clearance values per kilogram were equal or slightly lower in obese as compared with non-obese patients.

In conclusion, these studies indicate higher tubular secretion in obese as compared with non-obese individuals. To date, no information is available on the impact of obesity on the tubular secretion of drugs in children.

Table 13 Tubular secretion-mediated clearance in both obese and non-obese patients (pts)

Substrate (reference) ^a	Clearance						Refer- ence		
	Obese pts ^b	Non-obese pts ^b	Dose	Parameter	Obese pts ^b	Non-obese pts ^b		Significance	Weight normalized clearance (obese vs non-obese pts) ^{b,c}
Procainamide ¹⁶⁸	n = 7 TBW 100 (17) kg	n = 7 TBW 68 (12) kg	300 mg IV	CL	4.19 (1.13) mL/min ^d	2.68 (0.85) mL/min ^d	0.05 > p > 0.02	0.04 vs 0.04 mL/ min/kg	127
Ciprofloxacin ¹⁶⁹	n = 17 TBW 111 (20) kg	n = 11 TBW 72 (10) kg	400 mg IV	CL	638 mL/min	495 mL/min	p < 0.05	5.74 vs 6.88 mL/ min/kg	170
Cisplatin ¹⁷¹	n = 23 BMI <30 kg/m ²	n = 165 BMI <30 kg/m ²	50–100 mg/m ²	CL	60 L/h	53.3 L/h	p = 0.007	NA	59
Topotecan ²¹²	n = 21 BMI >30 kg/m ²	n = 108 BMI <30 kg/m ²	Protocol based	CL	21.7 L/h	19.6 L/h	p = 0.50	NA	59
Digoxin ²¹³	n = 13 TBW 100 kg	n = 16 TBW 65 kg	0.75 mg IV	CL	328 mL/min	278 mL/min	T-value 1.59 (NS)	3.28 vs 4.28 mL/ min/kg	172

a The references mentioned with the substrate (first column) refer to literature in which the appropriateness of the particular drug as a tubular secretion probe was confirmed.

b Unless otherwise specified, mean values (standard deviation).

c See section Approach for calculation of weight-normalized clearance values.

d Corrected for CL_{cr}.

BMI = body mass index; CL = clearance; CL_{cr} = creatinine clearance; IV = intravenously; NA = not available; NS = not significant; TBW = total body weight.

Table 14 Tubular reabsorption-mediated clearance in both obese and non-obese patients (pts)

Substrate (reference) ^a	Obese pts ^b	Non-obese pts ^b	Dose	Clearance					Reference
				Parameter	Obese pts ^b	Non-obese pts ^b	Significance	Weight normalized clearance (obese vs non-obese pts) ^{b,c}	
Lithium ²¹⁴	n = 10 TBW 110 (29) kg	n = 8 TBW 63 (7) kg	31.4 mEq	CL	33.9 (7.0) mL/min	23.0 (6.2) mL/min	p = 0.005	0.31 vs 0.37 mL/min/kg	173

a The references mentioned with the substrate (first column) refer to literature in which the appropriateness of the particular drug as a tubular reabsorption probe was confirmed.

b Unless otherwise specified, mean values (standard deviation).

c See section Approach for calculation of weight-normalized clearance values.

CL = clearance; mEq = milliequivalents; TBW = total body weight.

Tubular reabsorption

Studies on the influence of obesity on the tubular reabsorption of drugs are scarce (Table 14). Tubular reuptake of lithium in obese patients was reported to be lower, as lithium clearance was significantly increased in obese patients and glomerular filtration did not differ between these obese and non-obese patients¹⁷³. In contrast, proximal tubular reabsorption of sodium in obese patients is reported to be increased because of glomerular hyperfiltration¹⁷⁴.

Summary of renal elimination

The reviewed studies show that clearance of renally eliminated drug is higher in obese patients because of increased glomerular filtration and tubular secretion. The influence of obesity on the tubular reabsorption is unknown, as there is a lack of evidence on this topic.

DISCUSSION AND CONCLUSIONS

In this review, we have summarized the effects of obesity on drug metabolism and elimination. Studies that investigated pharmacokinetics of drugs in both obese and non-obese individuals were classified according to the drug's most important metabolic or elimination pathway. This allowed us to structurally review the influence of obesity on each individual metabolic or elimination pathway. Metabolic processes were subdivided into phase I metabolism, phase II metabolism and liver blood flow-dependent metabolism. Renal elimination was subdivided into glomerular filtration and tubular processes.

The reviewed studies show that the impact of obesity on drug metabolism and elimination differs greatly, depending on the metabolic or elimination pathway primarily involved in the handling of the investigated drug. In particular, CYP3A4-mediated drug elimination was found to be consistently lower, while UGT-, CYP2E1-, arylamine *N*-acetyltransferase type 2- and xanthine oxidase- mediated drug metabolism was consistently higher among obese as compared with non-obese subjects. Clearance mediated by phase I metabolizing enzymes CYP1A2, CYP2C9, CYP2C19 and CYP2D6 show trends towards higher clearance values in obese individuals.

Studies on drug clearance mediated by liver blood flow are somewhat inconclusive, although, on the basis of a few highly extracted drugs, an increase in liver blood flow can be noted in obese patients.

Regarding drug elimination, the reviewed studies show an increase of glomerular filtration and tubular secretion in obese patients. The influence of obesity on tubular reabsorption is unknown.

Many of the observed trends were also reflected in weight-normalized clearance values, which were halved (e.g. CYP3A4), almost equal (e.g. CYP2E1) or slightly decreased in obese as compared with non-obese individuals (e.g. CYP2C9 and tubular secretion). For other drug clearance pathways, trends in body weight-normalized clearance were not as pronounced (e.g. the glomerular filtration rate and CYP1A2). It should be emphasized that these body weight-normalized clearance values may provide information on quantitative differences in clearance values but do not explain the relationship between total body weight and drug clearance values.

The large number of studies included in this review shows that there is a substantial amount of information available on the impact of obesity on drug metabolism and elimination. However, in many of these studies, the difference in body weight between obese and non-obese subjects is rather small. More specifically, the obese subjects included in the reviewed studies are not as obese as the patients currently seeking medical care. From this perspective, information on drug metabolism and elimination in morbidly obese patients (BMI >40 kg/m²) and super-obese patients (BMI >50 kg/m²) is largely lacking and requires future research.

Regarding obesity in children, only five studies investigated pharmacokinetics of a drug in obese versus non-obese children, of which four were recently published^{78,92,117,132}. Regarding renal elimination, no pharmacokinetic studies of obese versus non-obese children were found. Extrapolation of results from studies in obese adults to obese children is widely applied because often no clinical studies in obese children are available^{24,175}. For the UGT mediated metabolism of paracetamol this may be justified, as paracetamol clearance in both adolescents⁷⁸ and adults⁷⁹ was increased. This strong similarity in results was not seen for other drugs that were studied in both adults and children such as caffeine⁹². Moreover, the expression and activity of enzymatic pathways in children

may be different compared with adults and are dependent on maturational status (age). In addition, obesity may influence the maturation process(es) itself, and the starting point of weight gain may also influence the maturation process(es), representing additional factors of variability in drug metabolism and elimination among obese adults and children. Taking this into consideration, extrapolation from adult observations may give false predictions of clearance values in children (and vice versa) and should be performed with care.

While it is impossible to study and assess the pharmacokinetics of every drug in obese subjects, future clinical trials should aim to quantify the impact of obesity on specific drug elimination pathways and on the underlying associated mechanisms (e.g. steatosis and inflammation). In this approach, study outcomes can be extrapolated to other drugs eliminated by the same pathway. This extrapolation can be achieved by using model drugs and within the context of a multidisciplinary research team including physicians, pharmacists, pharmacologists and pharmacometricians. Primarily, future research in this area should focus on individual metabolic and elimination pathways in adults and children that show increasing or decreasing trends in activity among obese versus non-obese individuals. As concluded from this review, these pathways include CYP3A4, CYP2E1, xanthine oxidase, UGT, *N*-acetyltransferase, glomerular filtration and tubular processes. Mainly, CYP3A4 deserves immediate research attention. Finally, particularly obese children and adolescents, and morbidly obese (BMI >40 kg/m²) and super-obese patients (BMI >50 kg/m²) should be included in these studies.

In conclusion, this systematic review of pharmacokinetic studies in obese and non-obese patients shows that the impact of obesity on drug metabolism and elimination greatly differs per drug metabolic or elimination pathway. However, the clinical trials reviewed here often only included overweight to moderately obese patients. As the prevalence of obesity and total body weights of both children and adults are still increasing and this trend will persist, future studies assessing the impact of morbid obesity on specific drug elimination pathways in both children and adults are warranted.

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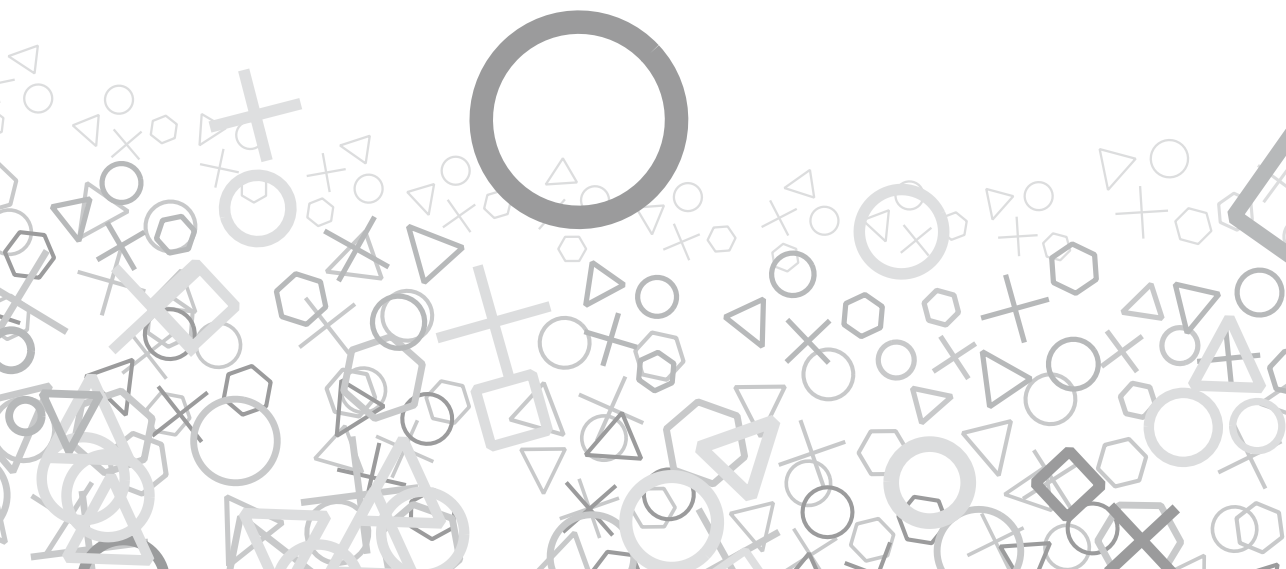


CHAPTER 3

Drug disposition in obesity: Toward evidence-based dosing

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ABSTRACT

Obesity and morbid obesity are associated with many physiological changes affecting pharmacokinetics, such as increased blood volume, cardiac output, splanchnic blood flow, and hepatic blood flow. In obesity, drug absorption appears unaltered, although recent evidence suggests that this conclusion may be premature. Volume of distribution may vary largely, but the magnitude and direction of changes seem difficult to predict, with extrapolation on the basis of total body weight being the best approach to date. Changes in clearance may be smaller than in distribution, whereas there is growing evidence that the influence of obesity on clearance can be predicted on the basis of reported changes in the metabolic or elimination pathways involved. For obese children, we propose two methods to distinguish between developmental and obesity-related changes. Future research should focus on the characterization of physiological concepts to predict the optimal dose for each drug in the obese population.

INTRODUCTION

Obesity represents a serious and increasing health problem worldwide. In the United States in 2009–2010, the prevalence of obesity (body mass index (BMI) > 30 kg/m²) was 35.9%, and the prevalence of morbid obesity (BMI > 40 kg/m²) was 6.3% (8.2% for women and 4.4% for men) ¹. Alarming, the prevalence of overweight and obesity in children is also increasing. According to the most recent National Health and Nutrition Survey (2009–2010) 31.8% of US children and adolescents (age 2–19 years) are overweight (≥85th percentile of BMI for age), 16.9% are obese (≥95th percentile), and 12.3% are morbidly obese (≥97th percentile) ². Worldwide prevalence rates for obesity in adults and overweight and obesity rates in children are also high, exceeding 24% in, for instance, Canada, Spain, the United Kingdom, Greece, Mexico, Saudi Arabia, Egypt, Australia, New Zealand, and some parts of South America ³.

Obesity increases the risk of many diseases and health conditions, such as hypertension, cardiovascular disease, dyslipidemia, type 2 diabetes, cancer, and osteoarthritis, thereby diminishing average life expectancy ⁴. In addition, obese individuals are also more likely to suffer from chronic pain ^{5–6} and nosocomial infections ^{7–8}. Because these comorbidities often require pharmacotherapeutic or surgical and anesthetic treatment, an important question is how to optimize the dose of drugs, particularly in light of the fact that the morbidly obese patient group is increasing. In this respect, specific attention should be paid to obese children, who are likely to become obese adults. Comorbidities associated with childhood obesity are hypertension, obstructive sleep apnea, diabetes mellitus, and coronary artery disease, necessitating pharmacotherapeutic or even surgical or bariatric treatment ^{9–10}. Furthermore, obese children are also more likely to develop asthma or severe asthma ¹¹, but their response to inhaled steroids is decreased ¹². Moreover, overweight and obesity have been reported as independent predictors of the relapse risk of acute lymphoblastic leukemia ¹³. It cannot be excluded that these differences result from changes in the pharmacokinetics of chemotherapeutic agents in overweight or obese children. Therefore, it is of utmost importance to gain insight into how to adjust the dose of drugs in obese and morbidly obese children and adolescents. This issue should be viewed through the perspective of the fact that even in nonobese children, 37–80% of drugs are prescribed in an off-label or unlicensed manner ^{14–16}.

In this review, we provide an overview of the current knowledge on changes in drug disposition in obese patients in relation to physiological changes associated with obesity. Our ultimate goal is to direct future research aiming for individualized dosing in this growing and heterogeneous patient population. We pay specific attention to changes in drug disposition in obese children.

PHYSIOLOGICAL CHANGES ASSOCIATED WITH OBESITY

Obesity is associated with many physiological and pathophysiological changes that may affect drug disposition. Obesity and morbid obesity are not only associated with an increase in fat but also in lean body weight (LBW), which is the weight devoid of all adipose tissue. The percentage of fat mass per kilogram of total body weight increases more than LBW in obese patients, with, for instance, an increase in LBW representing 20–40% of total excess of weight in morbidly obese patients¹⁷⁻¹⁸.

To supply the excess body mass with oxygen and nutrients, blood volume, cardiac output, and capillary flow increase substantially in obese and, in particular, morbidly obese individuals¹⁹⁻²². Serum albumin and total protein concentrations are reported to be comparable in lean and obese subjects, even though concentrations of alpha-1-acid glycoprotein are increased²³. In the cardiovascular system, the increased blood volume and cardiac output eventually leads to systemic hypertension, left and right ventricular hypertrophy, and an increased risk for sudden cardiac death due to conduction disorders²⁴⁻²⁵. Pulmonary function is uniformly altered in obesity, with reduced lung volumes²⁶ and a higher incidence of obstructive sleep apnea syndrome²⁷.

Nonalcoholic steatohepatitis and histological abnormalities such as fatty infiltration in the liver are very common in morbidly obese patients²⁸⁻²⁹. Because of the accumulation of fat in the liver of obese individuals, functional morphology may be altered owing to sinusoidal narrowing³⁰⁻³¹. However, because of increased blood volume and cardiac output, liver blood flow is not necessarily reduced in obese subjects³². Although liver volume is reported to be increased in obese individuals³³, the results of studies on the influence of obesity on expression and function of CYP enzymes are inconclusive, with the exception of CYP3A and CYP2E1; the expression and function of these enzymes have been reported to be decreased and increased, respectively³⁴.

There are conflicting data on alterations in renal function. Irrespective of the presence of hypertension, investigators have reported increases in glomerular filtration rate and effective renal plasma flow³⁵⁻³⁷. However, there is also evidence of unaltered renal function³⁸. In studies in Zucker rats with genetic obesity, researchers found that, after an initial increase in glomerular filtration rate, this rate normalized and subsequently decreased in the later stages of obesity, ultimately leading to end-stage renal disease³⁹⁻⁴¹. In morbidly obese patients who presented with proteinuria, one study reported focal glomerular sclerosis, diabetic nephropathy, or both⁴². In addition, estimates of the creatinine clearance from standard formulas tend to be inaccurate in obese patients⁴³⁻⁴⁵. Even though obesity-associated renal damage may be unpredictable, the available evidence indicates that it is best to use LBW in the Cockcroft-Gault formula for estimation of creatinine clearance in obese patients^{44,46}.

With respect to the functioning of the gastrointestinal tract, studies in obese subjects have found accelerated gastric emptying of solids⁴⁷⁻⁵⁰, high splanchnic blood flow¹⁹, and increased gut wall permeability⁵¹⁻⁵². Because studies on the influence of obesity on intestinal transit time and motility have shown contradictory results, the exact impact of obesity on drug or nutrient absorption remains unclear^{50,53-54}. Wisén & Johansson⁵⁴ found that obese subjects had significantly higher absorption in the proximal small intestine. Studies on the influence of obesity on enterohepatic recirculation are lacking.

MEASURES TO QUANTIFY BODY SIZE AND OVERWEIGHT

BMI is the international metric recommended by the World Health Organization to classify obesity⁵⁵. A BMI value between 18.5 and 25 kg/m² is considered healthy. BMI values greater than 30 and 40 kg/m² indicate obesity and morbid obesity, respectively⁵⁵. As BMI does not differentiate adipose tissue from muscle mass, BMI should be considered a descriptor of body shape instead of a measure of body composition⁵⁶⁻⁵⁷. For a child's weight status (2–18 years), an age- and sex-specific percentile for BMI (BMI-for-age) is used because children's body compositions vary as they age and between boys and girls⁵⁸⁻⁵⁹. For children younger than 2 years, weight-for-length charts are used. Overweight is defined as a BMI between the 85th and 95th percentile and obesity above the 95th percentage for children of the same age and sex⁶⁰.

The value of the ideal body weight (IBW) parameter is most commonly calculated using the equation by Devine⁶¹. Similar to BMI, this measure is rarely used as the basis for the individualization of drug dosage in obese patients, except for some specific drugs such as muscle relaxants⁶²⁻⁶⁴ and remifentanyl⁶⁵. This measure may lack predictive value for the dose adjustment of other drugs because it is based on height and sex only and does not consider body weight in any way⁵⁶. Adjusted body weight is an empirical, IBW-based metric with different correction factors (0.14-0.98) that was developed after the discovery that IBW was a suboptimal parameter for drug dosing in obese subjects⁶⁶, but very little evidence supports using this as a guide for dosing⁶⁷.

Body surface area (BSA) is mainly used for dosing of anticancer drugs, a practice that has a historical rather than scientific basis. BSA can be calculated using the equations by Dubois and Dubois⁶⁸ or Mosteller⁶⁹. The equations are based on the theory of Euclidean geometry and account for height and weight⁶⁶. Remarkably, recent reports have shown that there is no evidence to reduce the dose or dose capping when BSA-adjusted doses are used in obese or morbidly obese cancer patients⁷⁰⁻⁷¹. These results may be explained by the nonlinear relation of BSA with total body weight (Figure 1), reducing the absolute increase in dose in relation to the increase in body weight.

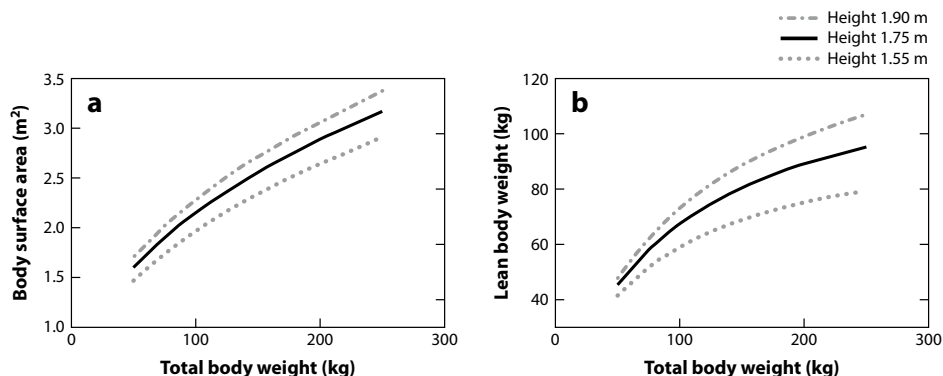


Figure 1 Lean body weight ⁷² (a) and body surface area ⁶⁸ (b) versus total body weight for males of various heights.

Because of the drawbacks of the previous measures, researchers have proposed using lean body weight (LBW) as a measure of body composition ⁷³. Information on body weight as well as height and gender are required to calculate LBW (Figure 1). LBW represents the weight of bones, muscles, tendons, and organs without body fat (i.e., fat-free mass). The most recent LBW equation, proposed by Janmahasatian *et al.* ⁷², provided good predictions of the fat-free mass as measured with bioelectrical impedance analysis or dual-energy X-ray absorptiometry. The exact value of LBW as a predictor for dosing remains to be established. In this respect, it is important to note that in pharmacometric studies, this parameter was not always identified as the best predictor ^{67,74-75}. Peters *et al.* ⁷⁶ proposed a new formula to calculate LBW in children. However, researchers have very limited experience with this measure as a predictor for dosing drugs in obese children ⁷⁷.

In general, actual body weight should be used with caution as a body-size descriptor in obesity because its value is influenced by factors such as age, sex, height, muscle mass, and obesity. Nevertheless, nonlinear functions of total body weight (TBW) show good performance as predictors of clearance in several pharmacokinetic studies covering wide ranges in body weight ^{74-75,78}. Similarly, in a large study on the variation in clearance and volume of distribution of 12 different drugs, total body weight appeared to be a consistent and reliable size descriptor for the prediction of these parameters in the obese ⁷⁹.

THE INFLUENCE OF OBESITY ON ORAL BIOAVAILABILITY AND ABSORPTION RATE

Only six studies have directly compared the oral bioavailability and absorption rate of drugs between obese and nonobese subjects on the basis of both oral and intravenous administration⁸⁰⁻⁸⁵. For propranolol, clearance (CL) after an intravenous dose was not different between six obese (136 ± 36 kg) and six control (67 ± 5 kg) subjects. However, oral clearance (CL/F) was lower in obese patients, indicating that the bioavailability (F) of propranolol was slightly higher for obese subjects ($35 \pm 4\%$ versus $27 \pm 2\%$, $p > 0.05$)⁸⁰. In the discussion of their article, the authors point out that the slightly higher bioavailability reported for propranolol may also be applicable for triazolam^{80,86}. Unfortunately, in the study on triazolam, there were no observations after intravenous administration⁸⁶, which makes it impossible to draw conclusions on an eventual difference in absolute bioavailability. For midazolam, no difference in bioavailability was found between normal-weight volunteers (66 ± 2 kg, $n = 20$) and obese volunteers (117 ± 8 kg, $n = 20$) ($40 \pm 3\%$ versus $42 \pm 4\%$, $p > 0.05$, respectively), nor was a difference found in time of maximum concentration ($T_{\max}$) or maximum concentration ($C_{\max}$) itself⁸¹. Similarly, no difference in bioavailability or oral absorption rate was found for trazodone, cyclosporine, dexfenfluramine, and moxifloxacin between obese and nonobese subjects⁸²⁻⁸⁵.

In view of the limited number of studies on oral absorption, we most recently studied midazolam bioavailability in 20 morbidly obese patients (mean body weight 144 kg (112 - 186 kg) and mean BMI 47 kg/m² (40 - 68 kg/m²)) and 12 healthy volunteers (76 kg (63 - 93 kg) and mean BMI 22 kg/m² (19 - 26 kg/m²)) (<http://clinicaltrials.gov/show/NCT01519726>). For this study, a semisimultaneous oral and intravenous administration design was chosen in which morbidly obese patients received 7.5 mg of midazolam orally followed by a 5 mg intravenous bolus dose after 159 ± 67 min. Healthy volunteers received 2-mg oral and 1-mg intravenous midazolam separated by 150 min. This study design allowed for the characterization of both clearance and bioavailability in a single pharmacokinetic study. Results of this study show an increased bioavailability ($60 \pm 13\%$ versus $28 \pm 7\%$, $p < 0.01$) and a lower oral absorption rate ($0.057 \pm 14\%$ min⁻¹ versus 0.13 ± 5 min⁻¹, $p < 0.01$), but no influence of obesity on systemic clearance in morbidly obese patients compared to healthy volunteers⁸⁷. Dose simulations of the final population pharmacokinetic model showed that after a 7.5-mg oral midazolam, $C_{\max}$ is only slightly lower, whereas $T_{\max}$ is increased for morbidly obese patients (Figure 2c).

The significant difference in oral bioavailability reported in this study⁸⁷ may result from the larger body weights of the subjects compared to the previous study by Greenblatt *et al.*⁸¹, who reported no difference in bioavailability (mean body weight of 144 kg versus 117 kg). The observed higher bioavailability could be explained by an increased splanchnic blood flow¹⁹, which may lead to reduced contact between midazolam and

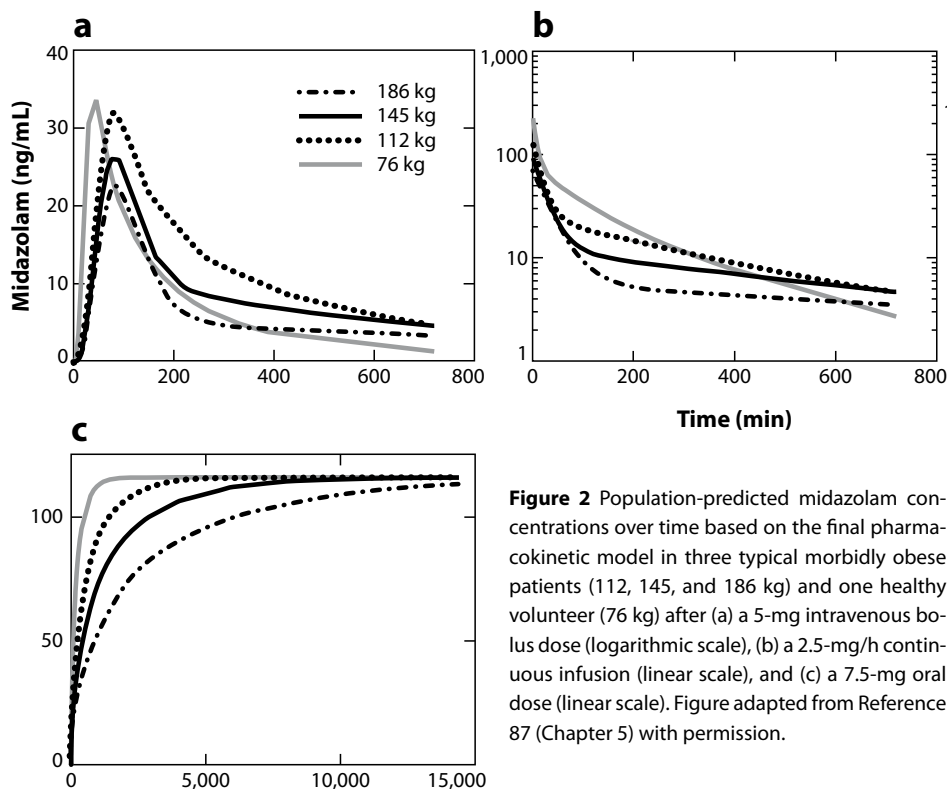


Figure 2 Population-predicted midazolam concentrations over time based on the final pharmacokinetic model in three typical morbidly obese patients (112, 145, and 186 kg) and one healthy volunteer (76 kg) after (a) a 5-mg intravenous bolus dose (logarithmic scale), (b) a 2.5-mg/h continuous infusion (linear scale), and (c) a 7.5-mg oral dose (linear scale). Figure adapted from Reference 87 (Chapter 5) with permission.

intracellular CYP3A enzymes in the gut wall. Also, the increase in bioavailability may be explained by increased paracellular absorption through the gut wall, or a combination of both^{51-52,88-89}. The higher midazolam bioavailability found in morbidly obese patients, however, does not seem to result in higher C_{max} values (Figure 2c); this may be explained by the higher volume of distribution⁸⁷ which was also reported by Greenblatt *et al.*⁸¹. The lower absorption rate (and therefore increased T_{max}) in morbidly obese patients may be the result of the difference in midazolam formulation, as healthy volunteers received an oral solution and morbidly obese patients a tablet. As midazolam effectiveness is determined by the initial midazolam concentrations after an oral dose, this study suggests that the net result of the alterations in the different pharmacokinetic parameters is that no adjustments in oral midazolam dose seem necessary for obese individuals. However, a different conclusion should be drawn for intravenous administration, given the substantially increased volumes of distribution of midazolam in morbidly obese patients (Figure 2a, b)^{81,87}.

There is limited information on the influence of obesity on drug pharmacokinetics after oral administration. This is a major limitation, given the fact that most drugs are

given orally. From the very small number of studies on drug absorption identified in this review, it seems that drug absorption is rather unaltered. However, this may be a premature conclusion warranting further systematic evaluations on drug absorption⁹⁰. Given the reported accelerated gastric emptying of solids⁴⁷⁻⁵⁰, increased splanchnic blood flow¹⁹, and increased gut permeability⁵¹⁻⁵² in obese subjects, changes in absorption rate and oral bioavailability cannot be excluded. The recent study on midazolam oral and intravenous pharmacokinetics in both morbidly obese patients and healthy volunteers confirms some of these anticipated changes⁸⁷. The design of this study may be used as an example to study drug absorption because both oral and intravenous administration were evaluated within each individual. Investigators analyzing results on drug absorption from a study without data after intravenous administration risk being unable to distinguish between the influence of obesity on clearance and bioavailability (or between volume of distribution and bioavailability). Finally, the consequences of altered absorption rate and oral bioavailability should each be evaluated for their clinical relevance and impact on drug dosing in the obese population.

THE INFLUENCE OF OBESITY ON DRUG DISTRIBUTION

Volume of distribution is an important parameter that is often substantially altered in obese patients^{79,90-92}. It is particularly important to characterize changes in volume of distribution when a rapid onset of the effect is needed as the peak concentration after single-dose administration is largely determined by the volume of distribution. The same applies for the time to reach steady state and an eventual loading dose as part of a continued or repeated administration scheme. A rapid onset of effect may be clinically relevant in anesthesia, for anticoagulation, and for antimicrobial drug effects.

In general, drug distribution depends on the physicochemical properties of the drug, such as molecular weight, lipid solubility, and protein binding, as well as the properties of the biological system^{91,93}. The latter properties may differ between subjects (obese subjects versus healthy volunteers). In obese subjects, changes in volume of distribution may be expected to result from increased blood volume, increased cardiac output and blood flow, increased LBW, increased adipose tissue and reduced tissue perfusion^{19-22,91-92}, with only a limited influence of changes in blood proteins (i.e., albumin, alpha acid glycoprotein)^{23,94}.

From the available evidence, the values of the volume of distribution appear highly variable in obese individuals and more difficult to predict than the values of clearance^{79,90}. While intuitively more influence of obesity on lipophilic drugs than on hydrophilic drugs may be expected⁹³, Jain *et al.*⁹⁰ concluded, on the basis of an overview of the ratios of volume of distribution of various drugs in obese versus nonobese individuals, that

changes in volume of distribution cannot be predicted on the basis of lipophilicity alone. More specifically, they showed that, for lipophilic drugs, the values for volume of distribution normalized with body weight may be increased, unchanged, or reduced⁹⁰. Also, in our experience, volume of distribution is difficult to predict. For instance, no influence of obesity on the peripheral volumes of distribution of propofol was observed, despite the high lipophilicity of the drug^{75,77-78}. For hydrophilic drugs, unchanged or decreased ratios of volume of distribution normalized with body weight were observed, but the magnitude of the effect of obesity was smaller than for lipophilic drugs⁹⁰.

Similarly, Mahmood⁷⁹ concluded, on the basis of a study on the pharmacokinetics of 12 different drugs, that predictions of volume of distribution in the obese from the values in normal-weight subjects were less accurate than predictions of clearance. Although total body weight appeared to be a more consistent and reliable size descriptor than other size descriptors for the prediction of volume of distribution⁷⁹, as was suggested before⁵⁶, linear scaling of volume of distribution with body weight was reported to lead to overprediction of volume of distribution in the obese for many drugs. Instead, prediction of volume of distribution by an allometric model on the basis of total body weight was more accurate. However, for the 12 drugs studied, the exponents of allometric functions were found to vary widely (0.27–2.459), illustrating the variability of changes in volume of distribution as a result of total body weight⁷⁹. As the allometric models were built on data from normal-weight subjects, Mahmood concluded that inclusion of data from the obese into these allometric models could lead to better predictions⁷⁹.

The relative impact of the obesity-related changes in volume of distribution with respect to adjusting the dose in obese individuals is illustrated below in three examples.

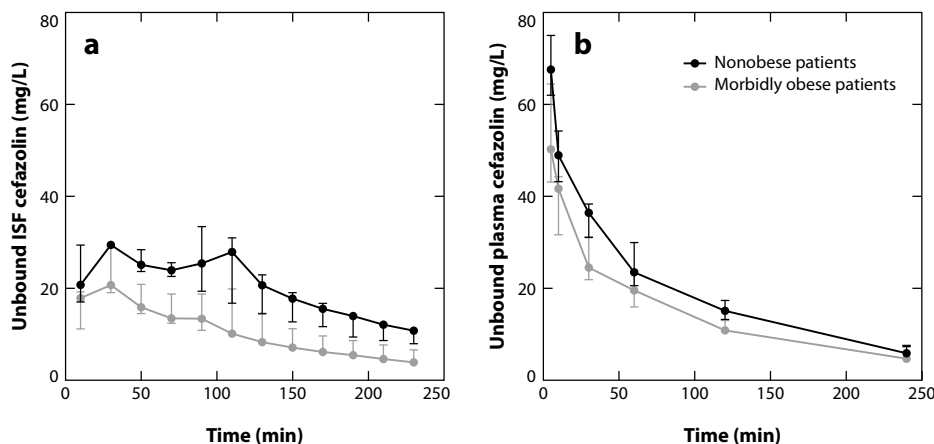


Figure 3 Concentrations of (a) subcutaneous interstitial space fluid (ISF) cefazolin and (b) unbound plasma cefazolin in morbidly obese (black, $n = 7$ for panel a and $n = 8$ for panel b) and nonobese (grey, $n = 7$ for both panels) patients. Figure adapted from Reference⁷⁴ (Chapter 4) with permission.

Example 1: Cefazolin

In a clinical microdialysis study, cefazolin concentrations in subcutaneous adipose tissue and in plasma were evaluated in morbidly obese and nonobese patients⁷⁴. Previously, no influence of morbid obesity was found on protein binding or on trough concentrations of cefazolin, whereas a modest influence of obesity was found on cefazolin peak concentrations upon an intravenous bolus administration⁹⁴. The results of the microdialysis study show that cefazolin penetration into the subcutaneous tissue over 4 h after dosing in obese patients was reduced by 30% on average (Figure 3).

These results were explained by reduced distribution of cefazolin to the subcutaneous tissue, which was found to depend on body weight, while there was no evidence for an increased peripheral volume of distribution represented by the subcutaneous tissue compartment⁷⁴. Instead, the value of the central volume of distribution was found to depend on body weight, and there was no influence of weight on clearance. Because time above the minimal inhibitory concentration at the target site is relevant for cefazolin prophylaxis, these findings have important consequences for the dosing regimen, particularly for the heaviest patients⁷⁴. In this respect, it is also important to take into account that obesity is an independent risk factor for postoperative surgical site infection^{7-8,95}.

Example 2: Nadroparin

A second example concerns anti-Xa levels, which Diepstraten *et al.*⁹⁶ measured to evaluate the effect of nadroparin in morbidly obese patients (107–260 kg). Prophylactic ranges have been defined for anti-Xa levels 4 h after subcutaneous dosing⁹⁷⁻⁹⁸. Volume of distribution is an essential parameter to determine the optimal dose for nadroparin. Upon subcutaneous administration, anti-Xa levels correlated best with LBW rather than BMI or total body weight, so dose adjustments on the basis of LBW are proposed⁹⁶.

An explanation for the finding that LBW should be used to dose low-molecular-weight heparins such as nadroparin could be that anti-Xa is a large, hydrophilic molecule that mainly distributes over vascular tissue and blood. Investigators have previously reported that blood volume increases with body weight in a nonlinear manner²², which probably corresponds to LBW. Also, researchers have proposed to adjust the dose for enoxaparin, another low-molecular-weight heparin, in obese individuals on the basis of LBW⁹⁹. Optimal dosing of low-molecular-weight heparins in obese individuals is particularly important because these individuals are at increased risk for venous thrombosis embolisms¹⁰⁰.

Example 3: Atracurium

As a third example, we present a pharmacodynamic study on atracurium in morbidly obese patients (BMI > 40 kg/m², body weight 112–260 kg)⁶². Patients were randomized to receive atracurium on the basis of IBW or total body weight (TBW). Dosing on the

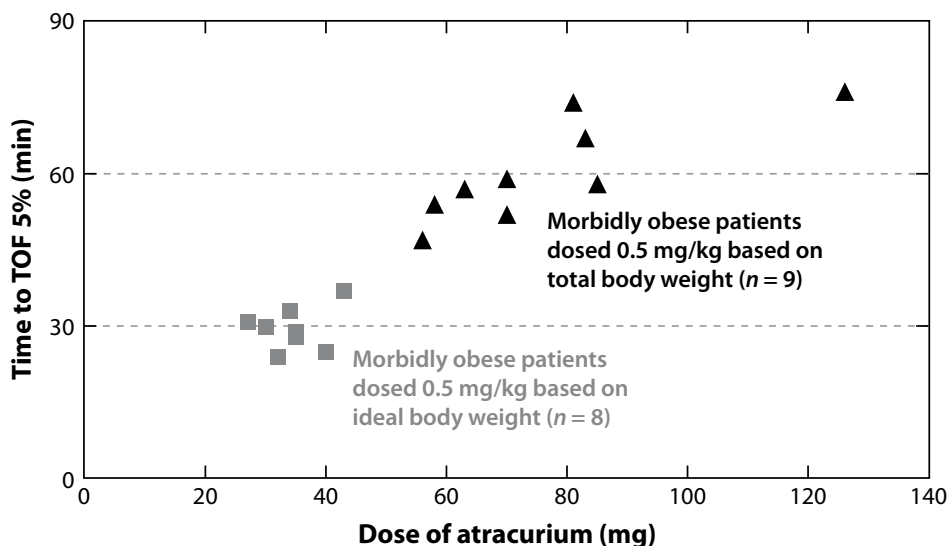


Figure 4 Effect of atracurium expressed as time to recovery of the twitch response of the neuromuscular train-of-four (TOF) to 5% versus dose for morbidly obese patients dosed 0.5 mg/kg based on ideal body weight (grey squares, $n = 8$) and dosed 0.5 mg/kg based on total body weight (black triangles, $n = 9$). Figure adapted from Reference ⁶² with permission.

basis of IBW resulted in a predictable profile of muscle relaxation, allowing for adequate intubation conditions and recovery of muscle strength within 60 min. In the patients for whom the dose was individualized on the basis of TBW, a dose-dependent prolongation of action was shown (Figure 4); thus, van Kralingen *et al.* ⁶² concluded that atracurium should be dosed on IBW.

In this example, changes in both pharmacokinetics (volume of distribution, clearance), and pharmacodynamics may have contributed to these results. Similar results have previously been reported for rocuronium ⁶³⁻⁶⁴. Remarkably, these results have led to an IBW-based dosing advice for rocuronium in the European label, whereas in the United States, rocuronium is still advised to be dosed on total bodyweight ⁹⁰.

From this overview, it seems that the current level of understanding of the comprehensive effect of obesity on volume of distribution is limited. Although volume of distribution often changes with obesity, the direction and magnitude is not always predictable ^{79,90}, despite many efforts to correlate it to physicochemical properties ^{17,90-92}. When no information is available, extrapolation on the basis of total body weight with an estimated allometric exponent from results in normal-weight subjects seems preferable ⁷⁹.

THE INFLUENCE OF OBESITY ON DRUG METABOLISM AND EXCRETION

Typically, there is more attention for the influence of obesity on metabolic and elimination clearance than on drug distribution^{79,101-103}. This may be explained by the fact that drug clearance is considered the most important pharmacokinetic parameter because it determines the maintenance dose of drugs.

A systematic review on reported clearance values of drugs in both obese and non-obese patients showed that the influence of obesity on drug metabolism and elimination differs between specific metabolic or elimination pathways¹⁰¹, even though the magnitude of its influence seems relatively small compared to the influence of obesity on distribution⁷⁹). Overall, the clearance of drugs primarily metabolized through the Phase II metabolism enzyme uridine diphosphate glucuronosyltransferase is reported to increase with obesity. For drugs that are eliminated through Phase I metabolism, the changes may differ depending on the pertinent enzyme. For example, an increased CYP2E1 clearance, a lower CYP3A clearance, and a trend toward higher clearance of CYP1A2, CYP2C9, CYP2C19, and CYP2D6 substrates have been reported¹⁰¹. In agreement with these literature findings, oral clearances were successfully predicted for eight drugs that are primarily cleared by CYP3A, CYP1A2, CYP2E1, and CYP2C9 on the basis of physiologically based pharmacokinetic modeling, in which known alterations in physiology resulting from obesity are implemented¹⁰³. More specifically, seven out of nine cases (involving eight drugs) were within 2-fold of the actual ratio between clearance in obese and lean patients¹⁰³. Remarkably, in this study, oral clearances of the CYP3A substrates alprazolam, midazolam, triazolam, and cyclosporine in the obese were somewhat over-predicted compared to observed oral clearance values, which were expected to be lower in the obese¹⁰³. As for midazolam, similar systemic clearance and higher bioavailability in morbidly obese patients were recently reported⁸⁷; it is emphasized that oral clearance equals CL/F and that reported differences in oral clearance in the obese may result from differences in systemic clearance, bioavailability, or both. Therefore, investigators should take care to predict systemic clearance on the basis of oral data as long as limited information is available on drug absorption in the obese.

With respect to renal clearance, higher values are reported in obese individuals^{35,101}. Recent results on the renally excreted antibiotic cefazolin in morbidly obese patients undergoing bariatric surgery did not identify an influence of body weight on cefazolin clearance, however^{74,94}. Even though this finding may be an artifact resulting from the relatively short sampling time in the study, a lack of change in glomerular filtration rate in obese individuals without microalbuminuria has been reported before³⁸, emphasizing that renal clearance of drugs may not necessarily be increased.

Concerning drug clearance mediated by liver blood flow, higher values were reported for a small number of high-extraction-ratio drugs with clearance values of more than 1.5 L/min¹⁰¹, which confirm early reports on increased hepatic flow in obese patients¹⁹.

Recently, Mahmood⁷⁹ has used an allometric equation to scale the pharmacokinetics of 12 drugs that are eliminated through different routes between healthy normal-weight subjects and obese patients. The results of this study indicate that clearances of these 12 different drugs increase in a nonlinear manner with total body weight⁷⁹, confirming a previous report⁵⁶. Clearance in the obese could be predicted with accuracy from normal-weight subjects using total body weight and simple allometry if an allometric exponent was estimated within the normal-weight population⁷⁹. In addition, allometric scaling with a fixed exponent of 0.75 or 1.0 was found to be inferior to the allometric model in which the exponent was estimated. Mahmood⁷⁹ also states that obesity may not have an impact on clearance at all, as was the case for phenazone, carbamazepine, lithium, remifentanyl, cefazolin, and theophylline; thus, we emphasize that allometric scaling using a fixed exponent of 0.75 or 1.0 on the basis of results from normal-weight patients should not be applied unless more data become available. This argument also applies to the proposal to scale clearance with LBW with an exponent of 2/3, independent of the drug's primary route of metabolism and elimination¹⁰², as this approach assumes an increase in clearance with obesity, which may not be the case for all drugs^{79,104}.

In conclusion, for clearance, the influence of obesity seems smaller and somewhat easier to predict compared to alterations in volume of distribution, even though many questions remain on the exact quantification¹⁰¹. From the results presented here, it seems that predictions can be made on the basis of the primary pathway involved^{101,103}. When no information is available, extrapolation on the basis of total body weight with an estimated allometric exponent from results in normal-weight subjects seems preferable⁷⁹.

CHARACTERIZATION OF THE INFLUENCE OF OBESITY IN CHILDREN

Despite the increasing numbers of obese and morbidly obese children, very limited pharmacokinetic and dosing information in obese children is available¹⁰⁵⁻¹⁰⁷. A specific aspect that investigators, regulators, and prescribers should consider when determining dosing guidelines for obese children and adolescents is that, in general pediatric practice, dosing regimens are expressed in mg/kg. This linear mg/kg-based dosing is subject to debate even in normal-weight children between 0 and 18 years¹⁰⁸⁻¹¹², but an overdose may be anticipated if the dosing is based on mg/kg total body weight in overweight and, particularly, obese and morbidly children. This underscores the need to develop dedicated models for obese and morbidly obese children and adolescents⁷⁸. Performing these studies in the target population of obese individuals is even more

relevant given that differences in pharmacokinetics, pharmacodynamics, or even the disease itself may exist in this population¹²⁻¹³.

In view of the limited number of pharmacokinetic studies in obese children^{101,113-114}, we present two pharmacokinetic studies in which data from overweight and obese children (and adults) of a large age range, along with their controls, are analyzed. In obese children, total body weight can be considered to be composed of both weight resulting from growth and development and weight from varying levels of obesity. This raises the question of, for instance, whether an obese 9-year-old child weighing 60 kg -in whom part of this body weight is physiological weight, i.e., body weight conforming to his age, and the other part is overweight- should receive the same dose as a normal-weight 16-year-old individual of the same weight. The distinction between physiological weight and overweight should be kept in mind when weight is studied as a covariate in children of varying ages and varying degrees of obesity.

Example 1: Propofol

For propofol, researchers performed a population pharmacokinetic meta-analysis with data from morbidly obese adults, adolescents, and children and their nonobese controls (body weight 37–184 kg, age 9–79 years)⁷⁷. In this analysis, propofol clearance was found to increase with body weight according to a power function. Age was identified and implemented as a second covariate using a bilinear function with two distinct slopes, reflecting an initial increase and, at the age of 41 years, a subsequent decrease in clearance (Figure 5).

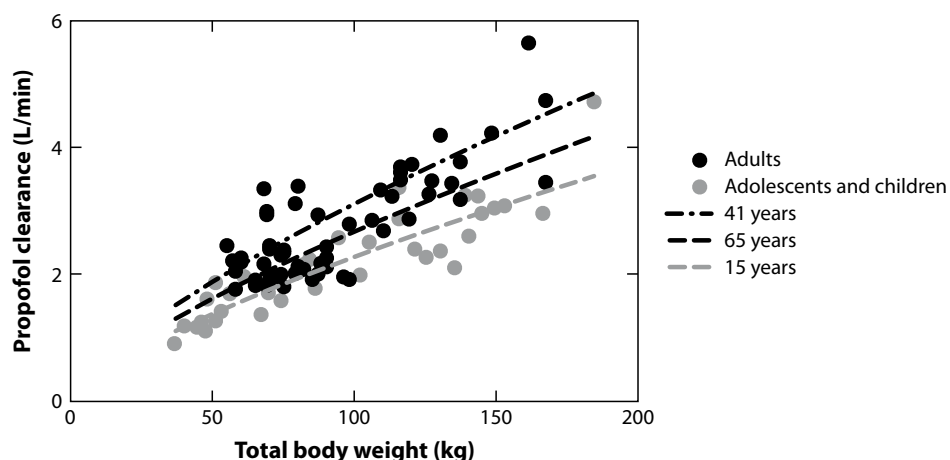


Figure 5 Individual post hoc propofol clearance estimates versus total body weight for morbidly obese adults and their nonobese controls (*black circles*) and obese adolescents and children and their nonobese controls (*grey circles*) ($n = 94$). The dashed lines indicate the population clearance values for 15, 41, and 65 years. Figure adapted from Reference⁷⁷ with permission.

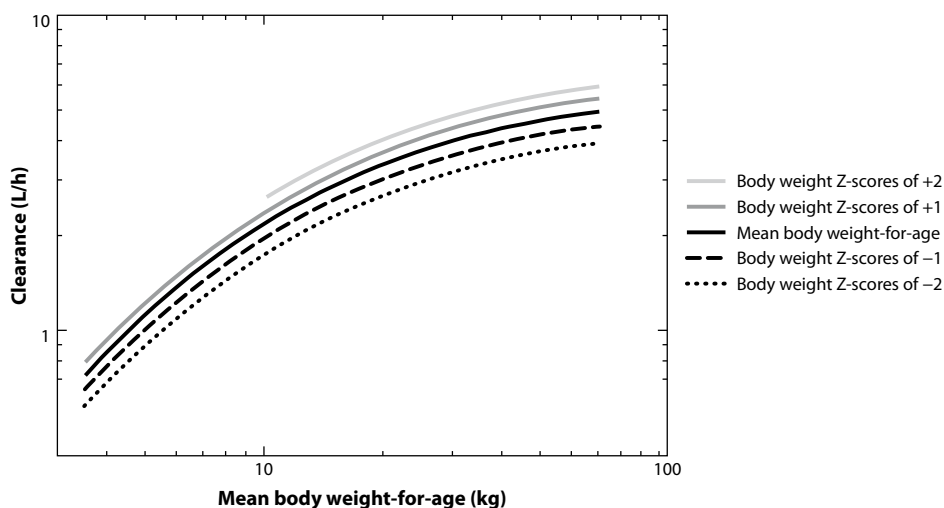


Figure 6 Busulfan clearance versus mean body weight-for-age for an exploratory model of overweight and underweight for children of all ages. In the model, a function for body weight due to growth (described using mean body weight-for-age, *black line*) and a function for body weight due to under- and overweight (described using the body weight Z-score) were implemented. Grey lines represent body weight Z-scores of +1 (*dark grey*) and +2 (*light grey*), and broken lines represent body weight Z-scores of -1 (*dashed*) and -2 (*dotted*). Figure adapted from Reference ¹¹⁵ with permission.

Example 2: Busulfan

In another study, investigators determined busulfan concentrations from a large population of underweight, normal-weight, and overweight children, adolescents, and adults (0.1–35 years) ¹¹⁵. This study used a previously derived, body weight-driven, pharmacokinetic model for busulfan in children of all ages ¹¹⁶. The results showed that the derived model ¹¹⁶ proved equally predictive in normal-weight, underweight, and overweight children ¹¹⁵. In addition, Bartelink *et al.* developed an exploratory model in which the body weight of each patient was considered to be composed of two parts: (a) physiological body weight related to growth (mean body weight-for-age) and (b) overweight, i.e., body weight related to under/overweight for a certain age (body weight Z-score) (Figure 6). Despite adequate performance of this exploratory model in which weight as a result of growth and obesity was disentangled (Figure 6), the model was not superior over the simple, weight-based model ¹¹⁵⁻¹¹⁶.

To capture the entire developmental change in clearance across the pediatric age range, this pharmacokinetic analysis of busulfan in over- and underweight children of all ages used an advanced power function based on body weight in which the exponent was allowed to change with body weight ¹¹⁶⁻¹¹⁷. This advanced power function was needed because very young infants were also included in the busulfan analysis, whereas the propofol analysis did not consider children younger than 9 years of age ⁷⁷. When this function was used for busulfan, the data were adequately described, and no influence of

age could be identified. In contrast, for propofol, a bilinear, age-based function with two distinct slopes was found (Figure 5)⁷⁷. The reason for this difference may be in part that, for the busulfan analysis, no patients above 35 years were included¹¹⁵. For busulfan, these results imply that within the ranges of age and weight studied, dosing in children can be based on actual body weight, irrespective of the level of over- or underweight¹¹⁵⁻¹¹⁶.

In conclusion, although very limited pharmacokinetic and dosing information is available in obese children¹⁰⁵⁻¹⁰⁷, we present two approaches on how to analyze data from children varying in age and degree of obesity (Figures 5 and 6). Future clinical studies should focus on the pharmacokinetics and pharmacodynamics of commonly used drugs in obese and morbidly obese children and adolescents to expand our knowledge in this clinically important area. Such studies should perform proper evaluations of the exact influence of weight resulting from growth, obesity, and age; these evaluations may be complicated because of the interrelation between weight and age in different manners, and they should use advanced validation frameworks, such as those described for pediatric pharmacokinetic analyses¹¹⁸.

PERSPECTIVES

To predict the optimal dose for each drug in the obese, not only well-designed clinical studies on drug disposition in obese adults and children upon oral and intravenous administration are needed. Future research should also focus on the characterization of physiological concepts that can be used across drugs. From this overview, it is clear that for none of the parameters bioavailability, volume of distribution or clearance, a general covariate model with one size descriptor and one allometric exponent can be defined without paying attention to the nature of the compound involved, including the route of elimination. In this respect, physiologically based modeling principles that take into account both drug characteristics and physiological changes in the obese body are of large importance.

For obesity-related changes in clearance, a recently reported, semiphysiological approach applied in children, in which information for one drug was used to predict changes for another drug sharing the same metabolic or elimination pathway, may deserve attention. Using this approach, the maturation function for glucuronidation of morphine in young children¹¹⁹⁻¹²⁰ was found to adequately predict the maturation in zidovudine glucuronidation in infants¹²¹. As the physicochemical drug parameters were not found to affect this maturation profile, researchers concluded that this maturation function for glucuronidation can also be used for other substrates of this enzyme¹²². This approach of between-drug predictions was also applied to renally excreted drugs in 0.5–5 kg neonates on the basis of a model derived for amikacin¹²³. This model has

recently been extended to older children and adults ¹²⁴ to obtain adequate predictions for other renally excreted drugs ¹²⁵⁻¹²⁶.

To predict volumes of distribution in the obese, investigators need to take into account both physicochemical properties and physiological changes in the obese body. Most recently, a new covariate relation that integrates body weight and LBW as covariates, with a weighting factor depending on the physicochemical properties of the drug, was proposed to predict volume of distribution at steady state ¹²⁷. Even though this approach was applied to only a limited number of obese individuals weighing below 100 kg, it deserves further exploration in the obese population, particularly because this approach to covariate modeling led to similar results as a whole-body, physiologically based pharmacokinetic model ¹²⁷.

CONCLUSION

In conclusion, although studies are particularly needed on absorption and distribution of drugs in obese individuals, some insight has been gained into changes in important metabolic and elimination pathways in obesity. For obese children, investigators need to perform clinical studies for which the proposed models ^{77,115} can be used to analyze the data. Future research should focus on the characterization of physiological concepts to predict the optimal dose for each drug in the obese.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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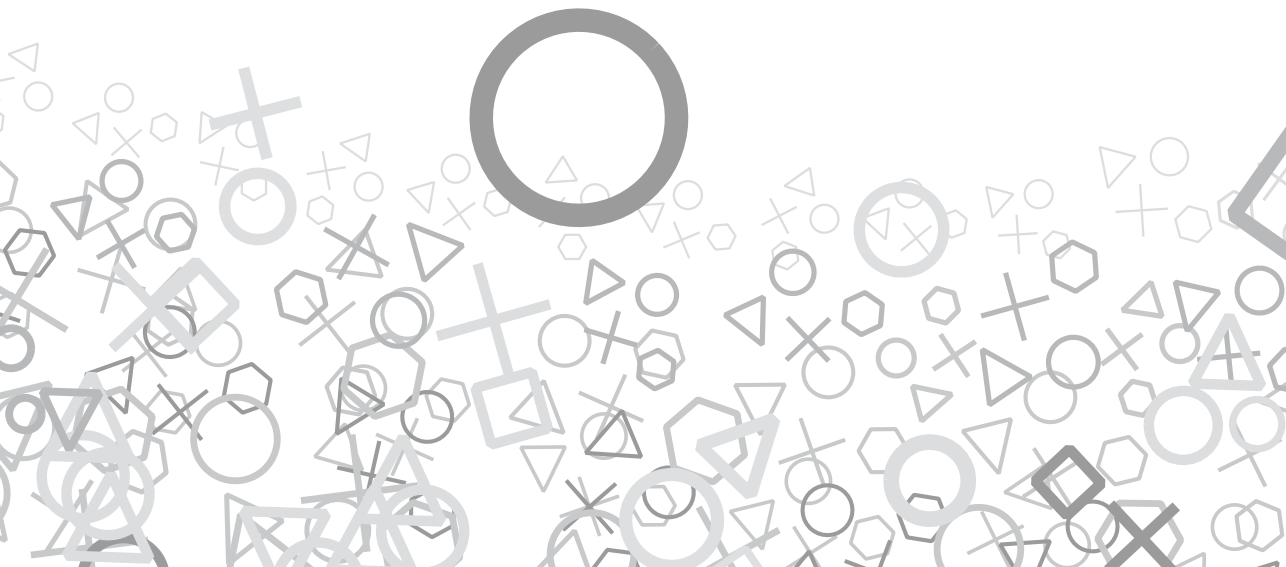
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Influence of morbid obesity on cefazolin pharmacokinetics



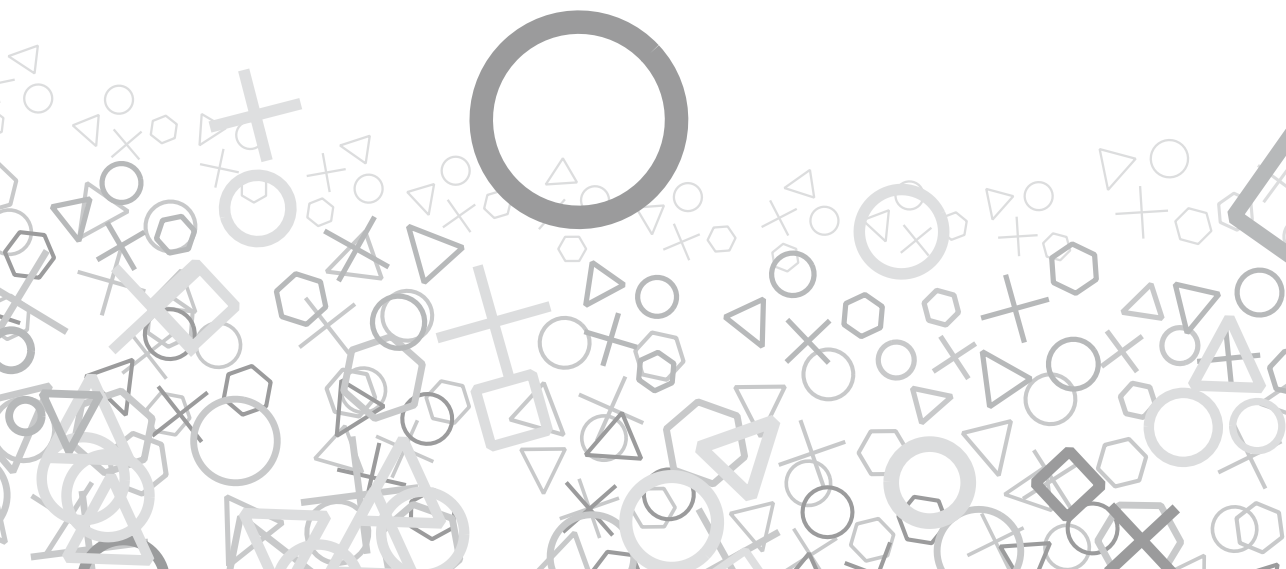


CHAPTER 4

Reduced subcutaneous tissue distribution of cefazolin in morbidly obese versus non-obese patients determined using clinical microdialysis

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ABSTRACT

Objectives

As morbidly obese patients are prone to surgical site infections, adequate blood and subcutaneous tissue concentrations of prophylactic antibiotic agents during surgery are imperative. In this study, we evaluated cefazolin subcutaneous adipose tissue distribution in morbidly obese and non-obese patients, thereby quantifying the influence of morbid obesity on cefazolin pharmacokinetics and enabling Monte Carlo simulations for subsequent dose adjustments.

Methods

Nine morbidly obese patients (body mass index (BMI) of 47 ± 6 kg/m²) of which eight were evaluable, and seven non-obese patients (BMI of 28 ± 3 kg/m²) received cefazolin 2 gram intravenously before surgery (NCT01309152). Using microdialysis, interstitial space fluid (ISF) samples of subcutaneous adipose tissue were collected together with total and unbound plasma cefazolin samples until 240 min after dosing. Using NONMEM, population pharmacokinetic modelling, covariate analysis and Monte Carlo simulations were performed.

Results

The unbound (free) cefazolin ISF penetration ratio ($fAUC_{\text{tissue}}/fAUC_{\text{plasma}}$) was 0.70 (0.68-0.83) in morbidly obese patients versus 1.02 (0.85-1.41) in non-obese patients ($p < 0.05$). A two-compartment model with saturable protein binding was identified in which the central volume of distribution and cefazolin distribution from the central compartment to the ISF compartment proved dependent on body weight ($p < 0.001$ and $p < 0.01$, respectively). Monte Carlo simulations showed reduced probability of target attainment for morbidly obese versus non-obese patients for MIC values of 2 and 4 mg/L.

Conclusions

This study shows that cefazolin tissue distribution is lower in morbidly obese patients and reduces with increasing body weight, and that dose adjustments are required in this patient group.

INTRODUCTION

The prevalence of obesity (body mass index (BMI) $>30\text{kg/m}^2$) and morbid obesity (BMI $>40\text{kg/m}^2$) is increasing worldwide. European obesity prevalence rates range between 4 and 37%, while in the USA 36% of the population is obese and 5% is morbidly obese¹⁻². Obesity and morbid obesity are considered an independent risk factor for postoperative surgical site infection³⁻⁵. To prevent surgical site infection, for surgery above or including the duodenum, cefazolin is the prophylactic agent of choice⁶. As a target site for prophylactic antibiotics, distribution to the interstitial space fluid (ISF) of the subcutaneous adipose tissue should be considered. At least between opening and closure of the skin, the unbound cefazolin concentration in the ISF should be above the minimal inhibitory concentration (MIC) for the target micro-organisms⁷.

Despite extensive use of cefazolin as antibiotic prophylaxis, there is limited data available from controlled clinical trials in morbidly obese patients. Previous studies in morbidly obese patients have so far only reported cefazolin concentrations in biopsy samples taken from fat tissue, but these samples inadequately reflect unbound cefazolin concentrations in the ISF as biopsy samples provide average concentrations for combined intra- and extracellular compartments⁸⁻¹⁰. Furthermore, cefazolin is highly protein bound and thus only a relatively small part of the concentration is available for antibiotic effect. To date, clinical microdialysis is the only sampling technique that allows measurement of extracellular, unbound (i.e. active) drug concentrations in virtually any tissue and is hence suitable for measuring unbound cefazolin concentrations in the ISF of the subcutaneous adipose tissue¹¹⁻¹².

Therefore, the objective of this study is to measure and compare unbound cefazolin concentrations in the ISF of the subcutaneous adipose tissue of morbidly obese and non-obese patients, using a microdialysis technique. The results were used to quantify the influence of overweight on cefazolin pharmacokinetics by developing a model for total and unbound plasma cefazolin and unbound cefazolin in the ISF, which can be used for Monte Carlo simulations and subsequent dose adjustments.

MATERIALS AND METHODS

Patients

Morbidly obese patients (BMI $>40\text{ kg/m}^2$) undergoing laparoscopic gastric bypass surgery and non-obese patients (BMI $20\text{--}30\text{ kg/m}^2$ at the inclusion of the study) undergoing laparoscopic Toupet fundoplication surgery were considered for inclusion in the study. Patients were excluded from the study if they were pregnant, breastfeeding, suffered from renal insufficiency, had a known allergy to cefazolin or had an ejection fraction

below 35%. Before participation, all patients gave written informed consent. Laboratory values for evaluation of renal function were available after inclusion of the patient in the study. The study was approved by the local human research and ethics committee of Nieuwegein (VCMO), The Netherlands (NL33065.100.10) 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.

Study design and procedure

This was a prospective observational study (NCT01309152). For anesthesia, all patients received propofol/remifentanyl and received a 2 gram intravenous (iv) bolus injection of cefazolin at 15.6 ± 4.3 (range of 8-24) minutes before start of surgery. Up to 4 hours after the cefazolin dose, blood and subcutaneous ISF samples were collected. Arterial blood samples were drawn for the measurement of total and unbound plasma cefazolin, while subcutaneous adipose ISF samples were collected using clinical microdialysis. Three hours before surgery a microdialysis probe (CMA60, Microdialysis, Solna, Sweden) was inserted in the subcutaneous tissue of the right or left side of the abdomen. After a 20 minute baseline perfusion period with blank lactated Ringer's, the catheter was perfused with 5 mg/L cefazolin in lactated Ringer's solution for 40 minutes for calibration of the microdialysis catheter using the retrodialysis technique¹³. A sample was collected during the last 20 minutes of the retrodialysis procedure to calculate the recovery:

$$\text{Recovery (\%)} = 100 - (C_{\text{dialysate}} / C_{\text{perfusate}} \times 100) \quad (\text{Eq. 1})$$

where $C_{\text{dialysate}}$ is the cefazolin concentration in the dialysate leaving the probe and $C_{\text{perfusate}}$ is the cefazolin concentration in the perfusion fluid entering the probe¹⁴. The microdialysis recovery ratio was $27.1\% \pm 8.0$ for morbidly obese patients (n=9) and $27.4\% \pm 13.4$ for non-obese patients (n=7). To prevent cefazolin carry over from the retrodialysis procedure to the actual samples after the cefazolin iv dose, the microdialysis catheter was washed out with blank lactated Ringer's solution for at least 2 hours after calibration. At the time of cefazolin iv administration, microdialysis sample collection was started and samples were collected every 20 minutes until 4 hours after the dose. As a result, each collected microdialysis sample represented the average concentrations over a time span of 20 minutes. Throughout the whole procedure the microdialysis flow rate was kept at 2 $\mu\text{L}/\text{minute}$.

In non-obese patients, to determine total and unbound cefazolin concentrations in plasma, arterial blood samples were taken before and at 5, 10, 30, 60, 120 and 240 minutes after the cefazolin iv dose. In morbidly obese patients, arterial blood samples for total cefazolin concentrations in plasma, were taken before and at 10, 120 and 240 minutes after dose and samples for unbound plasma cefazolin were collected at 5, 10,

30, 60, 120 and 240 minutes after the cefazolin iv dose. Blood samples were centrifuged at 3000 RPM (1500 g) for 15 minutes at 4°C and plasma was collected. Both plasma and microdialysis samples were stored at -80°C until analysis.

Drug assay

Total and unbound cefazolin concentrations in plasma were determined using a validated reversed-phase HPLC method with UV detection at 254 nm (total plasma cefazolin concentrations) and 272 nm (unbound cefazolin plasma and microdialysis concentrations), based on a modification of the method of Kamani *et al.*, described previously¹⁵⁻¹⁶. In brief, a LiChrospher 100 RP-18 5 µm column was used for separation and the mobile phase, a mixture of 0.01 M acetic acid, acetonitrile and methanol (87.4/12/0.6, v/v/v), was eluted at 0.71 mL/min. Microdialysis samples were injected directly onto the HPLC column. The limit of detection and limit of quantification for unbound cefazolin concentrations in plasma and cefazolin in lactated Ringer's (microdialysis samples) were 0.3 and 1 mg/L, respectively. For total cefazolin concentrations in plasma, the limit of detection and lower limit of quantification were 1 mg/L and 5 mg/L, respectively.

Statistical analysis

The student's *t*-test was applied to test differences in demographic variables between the study groups. For cefazolin concentrations the nonparametric Mann-Whitney test was applied to test statistical differences between the groups. The observed area under the time-concentration curve from 0 to 4 hours after the dose (AUC_{0-4h}) was calculated for each patient separately, using the linear trapezoidal rule¹⁷. Outlying data was evaluated using Grubb's test for detecting outliers¹⁸. These statistical analyses were performed using IBM SPSS software, version 19.0.0.

Population pharmacokinetic analysis and internal validation

The population pharmacokinetic analysis was performed by means of nonlinear mixed effects modelling using NONMEM (version 6.2, release 1.1; GloboMax LLC, Hanover, MD, USA)¹⁹. S-Plus (version 6.2; Insightful Software, Seattle, WA, USA) with NM.SP.interface© version 05.03.02 (LAP&P, Leiden, The Netherlands) was used to visualize the data. Discrimination between different models was made by comparison of the objective function value (OFV, i.e. -2 log likelihood (-2LL)). A *p* value <0.05, representing a decrease of 3.84 in the OFV, 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) 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 model. The internal validity of the population pharmacokinetic model was assessed by the bootstrap re-sampling method using 250 replicates and normalized prediction distribution errors (NPDEs) ²⁰. Parameters obtained with the bootstrap replicates were compared with the estimates obtained from the original dataset. NPDE plots were checked for normal distribution characteristics and trends in the data errors.

Structural model

To describe all cefazolin concentrations (total plasma, unbound plasma and unbound subcutaneous ISF concentrations) a two-compartment model (ADVAN 6) was used. The model was parameterized in terms of the volume of distribution of the central compartment (V1), volume of distribution of the subcutaneous ISF compartment (V2), inter-compartmental clearance from the central compartment to the subcutaneous compartment (Q), clearance from the central compartment (CL) and the fraction unbound (FU), as depicted in Figure 1. The fraction unbound (FU) was modelled according to equation 2,

$$FU = (C_{total} - B_{max} - K_d) + \sqrt{\frac{(C_{total} - B_{max} - K_d)^2 + 4 \times K_d \times C_{total}}{2 \times C_{total}}} \quad (\text{Eq. 2})$$

where B_{max} is the maximal binding capacity, C_{total} is the total cefazolin plasma concentrations and K_d is the dissociation constant for cefazolin binding to albumin.

Intercompartmental clearance between the central and subcutaneous ISF compartment (Q) was equated to CL as both values were very similar and this improved goodness of fit plots of the non-obese patients ($p < 0.01$; decrease of 20 in Objective Function Value, (-20 Δ OFV)).

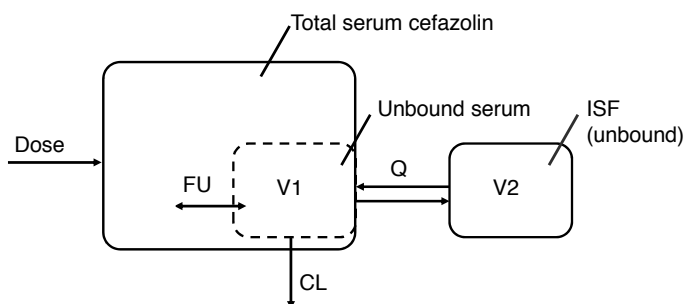


Figure 1 Schematic illustration of the population pharmacokinetic cefazolin model. The lines pointing towards a compartment indicate the type of observed data used for this compartment. CL= Clearance, FU= Fraction unbound, Q= Intercompartmental clearance, V1= Volume of distribution of central compartment, V2= Volume of distribution of subcutaneous compartment.

Statistical model

The individual parameter estimate (Empirical Bayes Estimate or *post hoc* value) of the *i*th individual was modelled according to (equation 3):

$$\theta_i = \theta_{mean} \times \exp^{\eta_i} \quad (\text{Eq. 3})$$

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.

The residual variability, resulting from assay errors, model misspecifications and other unexplained sources, was best described with a proportional error model for total and unbound cefazolin plasma concentrations and a separate proportional error for unbound subcutaneous ISF cefazolin concentrations. The *j*th observed cefazolin concentration of the *i*th individual (Y_{ij}) is described by equation 4:

$$Y_{ij} = C_{pred,ij} \times (1 + \epsilon_{ij}) \quad (\text{Eq. 4})$$

Where $C_{pred,ij}$ is the population predicted cefazolin 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 .

Covariate analysis

Covariates were plotted independently against the individual eta (η) estimates of pharmacokinetic parameters to visualize potential relations. The following covariates were tested: total body weight (TBW), BMI, lean body weight (LBW)²¹, sex, obesity and age. Covariates (except for sex and obesity) were tested using linear and allometric equations (equation 5 and 6):

$$P_i = P_p \times \left(\frac{COV}{COV_{median}} \right)^x \quad (\text{Eq. 5})$$

$$P_i = P_p \times (1 + Y \times (COV - COV_{median})) \quad (\text{Eq. 6})$$

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; X represents the exponential scaling factor, which was fixed at 1 for a linear function or an estimated value for a power function; and Y represents a correlation factor between the population pharmacokinetic parameters and the change in covariate value. The binary covariates sex and obesity were tested using the following equation:

$$P_i = P_p \times Z^{COV} \quad (\text{Eq. 7})$$

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 equaling 1. Potential covariates were separately entered into the model and statistically tested by use of the OFV and, if applicable, the 95% confidence interval of the additional parameter. In addition, if applicable, we evaluated whether the interindividual variability in the parameter concerned reduced in value upon inclusion of the covariate on the parameter. When more than one significant covariate for the simple model was found, the covariate-adjusted model with the largest decrease in the OFV was chosen as a basis to sequentially explore the influence of additional covariates with the use of the same criteria. Finally, after forward inclusion ($p < 0.05$), a backward exclusion procedure was applied to justify the inclusion of a covariate ($p < 0.01$). The choice of the covariate model was further evaluated as discussed above (in *Population pharmacokinetic analysis and internal validation*).

Monte Carlo simulations

Monte Carlo simulations based on body weight and age distributions of the original populations, were performed to simulate cefazolin concentration-time profiles of 5000 morbidly obese patients and 5000 non-obese patients. In these simulations, the unbound (free) area under the curve ratios ($fAUC_{\text{tissue}}/fAUC_{\text{plasma}}$) were calculated by allowing the unbound plasma and subcutaneous concentrations to accumulate over time in hypothetical compartments.

RESULTS

Patients and data

Nine morbidly obese patients with a mean body weight of 141.4 ± 22 kg (range 107 – 175) and 7 non-obese patients with a mean body weight of 86.2 ± 13 kg (range 72 – 109) participated in the study. Immediately after inclusion, one morbidly obese patient was excluded from the study because of an estimated glomerular filtration rate (GFR) of 60 mL/min instead of an estimated GFR >60 mL/min (ID 3), which was noticed after inclusion. Furthermore, ISF measurements from another morbidly obese patient were excluded from the analysis because the unbound area under the ISF curve ($fAUC_{\text{ISF } 0-4 \text{ h}}$) and $fAUC_{\text{tissue}}/fAUC_{\text{plasma}}$ ratio of this patient were strongly deviating and outlying based on the Grubb's test for detecting outliers¹⁸ ($p < 0.05$ and $p < 0.01$, respectively (ID 2)).

Patient characteristics of 8 morbidly obese and 7 non-obese patients are summarized in Table 1.

Table 1 Patient characteristics

	Morbidly obese	Range	Non-obese	Range	p value
Male : Female	1/7		4/3		
Age (years)	40.1 ± 5.5	(32-48)	53.7 ± 6.3	(42 - 61)	0.001
Body weight (kg)	140.4 ± 23	(107 -175)	86.2 ± 13	(72 - 109)	<0.001
Lean body weight (kg)	75.2 ± 8.5	(64 - 89)	55.5 ± 5.7	(48 - 62)	<0.001
BMI (kg/m ²)	47.0 ± 5.8	(41 - 57)	28.2 ± 2.8	(24 - 31)	<0.001
Surgery duration (min)	63.6 ± 12	(51 - 86)	59.6 ± 19	(39 - 92)	0.640
Wound closure post dose (min)	79.4 ± 14	(65 - 105)	74.1 ± 19	(55 -108)	0.557

Data are mean ± standard deviation and range (minimum-maximum).

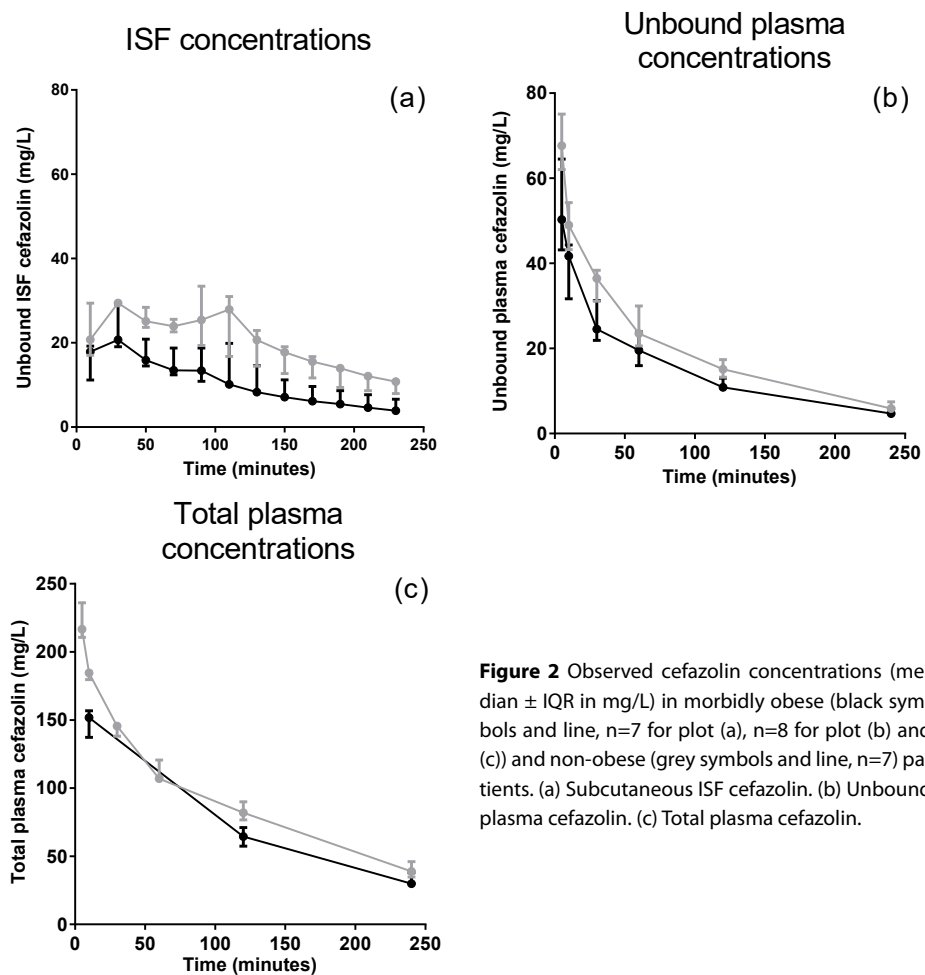


Figure 2 Observed cefazolin concentrations (median ± IQR in mg/L) in morbidly obese (black symbols and line, n=7 for plot (a), n=8 for plot (b) and (c)) and non-obese (grey symbols and line, n=7) patients. (a) Subcutaneous ISF cefazolin. (b) Unbound plasma cefazolin. (c) Total plasma cefazolin.

Observed cefazolin concentrations in plasma and ISF

Figure 2 shows median and interquartile ranges of observed cefazolin concentrations for morbidly obese and non-obese patients; panel (a) shows unbound cefazolin in the ISF of the subcutaneous adipose tissue, panel (b) shows unbound plasma concentration and panel (c) shows total plasma concentrations.

The area under the time- unbound concentration curve ($fAUC_{0-4\text{ h}}$) for subcutaneous ISF was significantly lower in morbidly obese patients ($n=7$) in comparison with non-obese patients ($n=7$), $p<0.05$. In contrast, the $fAUC_{0-4\text{ h}}$ for unbound plasma cefazolin concentrations did not differ significantly between the patient populations ($p>0.05$). The observed unbound cefazolin ISF penetration ratio, expressed as $fAUC_{\text{tissue}}/fAUC_{\text{plasma}}$ was 0.70 (range 0.68-0.83) in morbidly obese patients as opposed to 1.02 (range 0.85-1.41) in non-obese patients ($p<0.05$).

Population pharmacokinetic model and validation

A two-compartment pharmacokinetic model with saturable plasma protein binding best described the data (Figure 1, equation 2). Using this structural model without covariates, total and unbound plasma cefazolin concentrations in both patient groups were well described, while individual and population-predicted subcutaneous ISF concentrations were overpredicted in morbidly obese patients and underpredicted in the non-obese patients. Exploration and testing of covariates for V_1 , V_2 , Q , CL and B_{max} showed improvements of fit for unbound cefazolin plasma concentrations; however, the observed trend for subcutaneous ISF cefazolin concentrations (overprediction for morbidly obese patients, underprediction for non-obese patients) could not be explained by any of the preliminary covariates on any of the parameters. Therefore, potential nonlinearity in cefazolin distribution from the central (V_1) to subcutaneous ISF compartment (V_2) was evaluated by adding a power function (γ) on the cefazolin amount (concentration) in the central compartment (A_1):

$$\Delta A_1/\Delta t = -k_{12} \times A_1^\gamma \times FU - k_{10} \times A_1 \times FU + k_{21} \times A_2 \quad (\text{Eq. 8})$$

where A_x stands for the amount of cefazolin in the x th compartment, FU is the fraction unbound (equation 2) and k_{12} is a rate constant between compartments 1 and 2.

Although no nonlinearity was identified because gamma was not found to differ significantly from 1, addition of interindividual variability on gamma strongly improved the goodness of fit of the subcutaneous ISF concentrations ($p<0.001$, $-124 \Delta\text{OFV}$). Parameter values of the simple model without covariates are summarized in Table 2.

With the extended model, a covariate analysis was performed and exploratory plots of covariates against individual *post hoc* parameter estimates of the simple model showed potential relationships for different body size descriptors (TBW, BMI and LBW) with

Table 2 Population pharmacokinetic parameters of the simple and final pharmacokinetic model for ce-fazolin in morbidly obese and non-obese patients and results of bootstrap analysis (250/250 resamples successful).

Parameters	Simple model (CV)	Final model (CV)	Bootstrap (CV)
<i>Fixed effects</i>			
CL (L/min)	0.384 (7.5)	-	-
$CL = CL_{47 \text{ years}} * (AGE/47)^{-1}$			
$CL_{47 \text{ years}}$ (L/min)	-	0.371 (5.7)	0.371 (5.5)
V1 (L)	8.79 (5.1)	-	-
$V_1 = V_{109 \text{ kg}} * (1 + Y * (TBW - 109))$			
$V_{109 \text{ kg}}$ (L)	-	8.94 (3.0)	8.97 (3.1)
Y	-	0.0052 (18.0)	0.0051 (21.5)
V2 (L)	8.1 (8.9)	8.31 (7.8)	8.36 (7.6)
B_{max} (μM)	530 (7.8)	469 (7.3)	471 (7.6)
Kd (μM)	81.2 (11.4)	71.3 (10.9)	71.7 (11.2)
Gamma	1 fixed	-	-
$\text{Gamma} = \text{Gam}_{\text{pop}} * (TBW/109)^Z$			
Gam_{pop}	-	1 fixed	1 fixed
Z	-	-0.0946 (-27.6)	-0.0895 (-34.2)
<i>Interindividual variability (%)</i>			
CL	31.2 (28.1)	22.6 (31.6)	21.5 (32.9)
Bmax	20.6 (41.6)	11.6 (33.4)	10.8 (41.4)
Gamma	3.9 (28.9)	2.8 (50.4)	2.7 (53.2)
<i>Residual variability (%)</i>			
Total and unbound plasma	13.1 (18.2)	10.0 (22.2)	9.7 (22.1)
Subcutaneous ISF	16.5 (19.9)	17.0 (19.7)	16.7 (19.5)
OFV	1260.5	1166.5	1142.0 (9.7)

AGE= age in years, B_{max} = maximal plasma protein binding capacity, CL= Clearance (L/min), CV= coefficient of variation (%), Gamma= gamma factor or γ , power function on the cefazolin amount in the central compartment, Kd= dissociation constant for cefazolin protein binding to albumin, OFV= Objective function value (-2LL), TBW= total body weight (kg), V1= central volume of distribution (L), V2= subcutaneous interstitial space fluid (ISF) volume of distribution (L)

volume of distribution (V1) and the γ factor, for age and TBW with clearance and for LBW with B_{max} . After forward inclusion and backward deletion of covariates in the model, TBW proved to be the strongest predictor of interindividual variability of both central volume of distribution ($p < 0.001$, -77 ΔOFV) and γ representing cefazolin distribution to subcutaneous tissue ($p < 0.01$, -10 ΔOFV). For clearance, age significantly improved the model ($p < 0.01$, -10 ΔOFV). Finally, LBW seemed to be a covariate for B_{max} ; however, this covariate relationship was not included in the final model due to limited statistical significance ($p > 0.01$, -6 ΔOFV) in the backward deletion step.

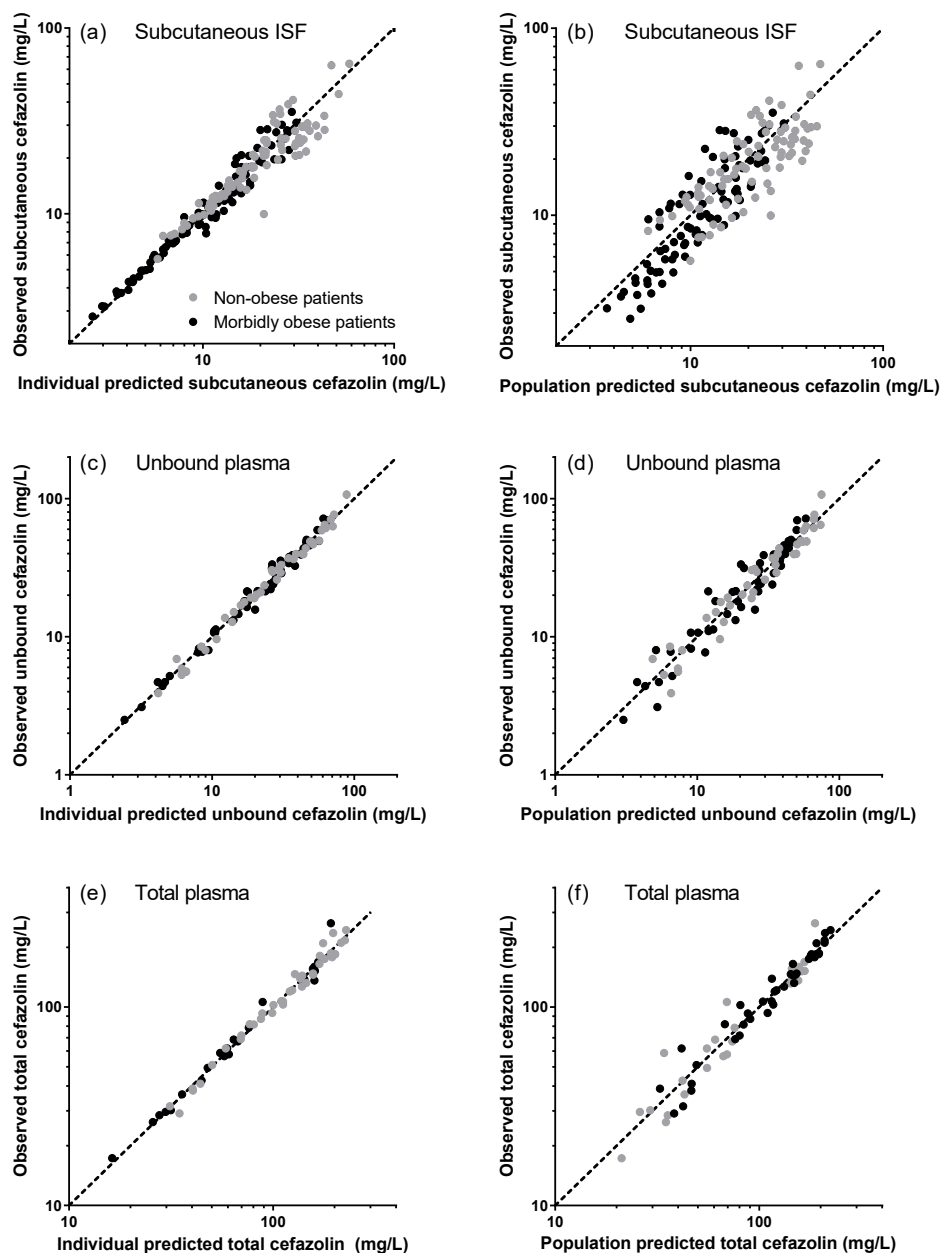


Figure 3 Observed versus individual predicted (a, c, e) and population predicted (b, d, f) cefazolin concentrations of subcutaneous ISF cefazolin (a and b), unbound plasma cefazolin (c and d) and total plasma cefazolin (e and f) in morbidly obese and non-obese patients. The dashed line represents the line of identity ($x=y$).

Parameters estimates of the final covariate model are summarized in Table 2. The table shows that implementation of the covariates age and total bodyweight on the parameters γ and clearance in the final model indeed explained variability in these parameters (decrease in interindividual variability in γ and clearance of 1.1 % and 8.6 %). Figure 3 shows observed versus population predicted cefazolin concentrations in the ISF of subcutaneous tissue (b), unbound plasma (d) and total plasma (f) for morbidly obese and non-obese patients of the final model. The figure shows that there was no remaining bias in any of the plots between data from morbidly obese or non obese patients, except for a slight overestimation of the lower subcutaneous concentrations in some of the morbidly obese patients (figure 3b).

Table 3 Probability of 5000 Monte Carlo-simulated morbidly obese and 5000 non-obese patients attaining cefazolin MIC targets of 1, 2 and 4 mg/L at 120, 180 and 240 minutes after a 2 gram iv cefazolin dose

	120 min post dose		180 min post dose		240 min post dose	
	Morbidly obese	Non-obese	Morbidly obese	Non-obese	Morbidly obese	Non-obese
ISF						
> 1 mg/L	1.00	1.00	1.00	1.00	0.998	1.00
> 2 mg/L	1.00	1.00	0.996	1.00	0.956	0.997
> 4 mg/L	0.996	1.00	0.909	0.995	0.663	0.949
Plasma						
> 1 mg/L	1.00	1.00	1.00	1.00	0.999	1.00
> 2 mg/L	1.00	1.00	0.999	1.00	0.965	0.994
> 4 mg/L	0.999	1.00	0.941	0.991	0.699	0.901

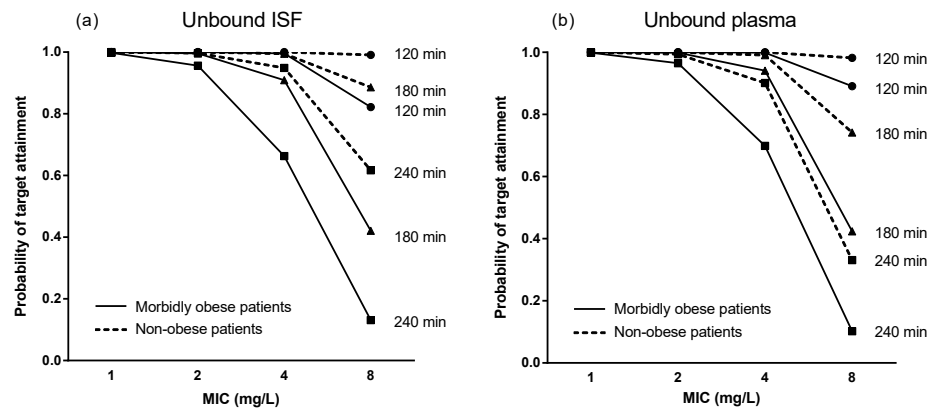


Figure 4 Probability of target attainment (PTA) at four different MIC values 120, 180 and 240 minutes after a 2 gram cefazolin iv dose in 5000 morbidly obese and 5000 non-obese Monte Carlo-simulated patients. (a) PTA of unbound cefazolin concentrations in the subcutaneous ISF. (b) PTA of unbound plasma cefazolin concentrations.

The final covariate model was validated using bootstrap analysis confirming the results (Table 2) and normalized predictions distributions errors analysis which indicated normal distributions of errors (Figure S1, available as Supplementary data at JAC Online and at the end of this Chapter).

Monte Carlo simulations

The final covariate model was used to simulate concentration-time profiles of subcutaneous ISF and unbound plasma cefazolin in 5000 morbidly obese and 5000 non-obese patients. The probability for the patient groups remaining above a certain minimal inhibitory concentration (MIC) 120, 180 and 240 minutes after a 2 gram iv dose are summarized in Table 3. Figure 4 illustrates the probability of target attainment that can be expected for unbound cefazolin concentrations in the ISF of morbidly obese versus non-obese patients. It shows that the probabilities of target attainment of unbound cefazolin plasma concentrations are more similar in both patient groups. The mean simulated unbound cefazolin ISF penetration ratio, expressed as $fAUC_{\text{tissue}}/fAUC_{\text{plasma}}$ for morbidly obese patients was 0.85 ± 0.19 in morbidly obese patients and 1.14 ± 0.27 in non-obese patients.

DISCUSSION

This study aimed to measure and compare unbound cefazolin concentrations in the ISF of the subcutaneous adipose tissue of morbidly obese and non-obese patients and to quantify the influence of overweight and other covariates on cefazolin pharmacokinetics. Using clinical microdialysis, it was found that unbound cefazolin subcutaneous tissue penetration was lower in morbidly obese compared with non-obese patients. When analyzing these results in a population analysis, a two compartment population pharmacokinetic model with saturable protein binding was found to adequately describe all measured cefazolin concentrations. The covariate analysis showed that central volume of distribution increased linearly with body weight and that cefazolin distribution from the central to subcutaneous compartment decreased with body weight in a nonlinear manner.

Unbound cefazolin concentrations in the ISF of the subcutaneous adipose tissue have not been reported previously for morbidly obese patients, despite the fact that reduced tissue penetration of antibiotic agents in morbidly obese versus non-obese patients has been reported before. Cefoxitin, which is a cephalosporin class antibiotic agent like cefazolin, also showed a reduced tissue penetration in morbidly obese versus non-obese patients (0.08 ± 0.07 versus 0.37 ± 0.26 , $p < 0.05$), although this AUC ratio was calculated using total cefoxitin plasma concentrations instead of unbound plasma concentrations, while cefoxitin is ~34% protein bound²². Also, in that study morbidly obese patients

were compared with mostly healthy volunteers who did not undergo surgery²². Furthermore, reduced tissue penetration in morbidly obese patients was found for ciprofloxacin (0.45 ± 0.27 versus 0.82 ± 0.36 , $p < 0.01$), though in the study by Hollenstein *et al.* protein binding was not considered either²³. The lower drug penetration into the subcutaneous adipose tissue of morbidly obese patients found in these studies and in the present study may potentially be explained by lower subcutaneous adipose blood flow. It has been shown before that subcutaneous adipose tissue blood flow in obese and morbidly obese patients is lower than in healthy control subjects²⁴⁻²⁵. Additionally, Joukhadar *et al.* found in healthy volunteers that enhanced subcutaneous blood flow resulted in higher subcutaneous ciprofloxacin concentrations²⁶. Therefore, we think that the lower subcutaneous adipose tissue penetration of cefazolin in morbidly obese patients after a single dose may be explained by lower subcutaneous adipose tissue blood flow.

In the population pharmacokinetic model the difference in subcutaneous ISF cefazolin concentrations in morbidly obese and non-obese patients was not adequately described by TBW on central volume of distribution (V1) alone or by additional covariates for intercompartmental clearance (Q) or the subcutaneous ISF compartment (V2). However, the introduction of interindividual variability on a γ factor (equation 8) on the distribution of cefazolin amount from the central (V1) to the subcutaneous compartment (V2) was able to improve the goodness of fit of the subcutaneous cefazolin data for both patient groups. Despite the small absolute difference in γ between a morbidly obese and non-obese patient, it strongly impacts on cefazolin distribution from the central to the subcutaneous compartment: where a non-obese individual of 75 kg with a corresponding γ value of 1.02 transports 300 mg unbound cefazolin/minute from the central to subcutaneous compartment, a morbidly obese patient of 145 kg with a corresponding γ value of 0.96 transports only 210 mg per minute (30% difference). For the final pharmacokinetic model, TBW on V1 and TBW on the γ factor were found to be the most predictive covariates for the reduced cefazolin distribution observed in morbidly obese patients. The slight overestimation of lower subcutaneous cefazolin concentrations in morbidly obese patient can be explained by the relatively high interindividual variability observed for cefazolin subcutaneous concentrations. While the model underpredicts concentrations at 230 minutes after dosing for some morbidly obese patients, for others it overestimates other concentrations at the same time after dose for others.

In contrast to the differences observed in cefazolin distribution between morbidly obese and non obese patients, we found that cefazolin saturable protein binding was similar for both patient groups. Plasma albumin concentrations were not measured in this study and may have been a covariate for maximal binding capacity (B_{max}). However, for this parameter interindividual variability was relatively small (11.6%) and thus the influence of difference in albumin concentration on cefazolin pharmacokinetics is assumed to be limited. Furthermore, the extent of saturable protein binding corresponded

to earlier reports in non-obese and morbidly obese patients^{16, 27-28}, and estimated $B_{\max}$ and K_d values correspond to values found in earlier studies in human plasma, in which $B_{\max}$ was reported to be 438 μM and K_d was 50 and 60.2 μM ²⁹⁻³⁰.

To determine the efficacy of prophylactic cefazolin, currently the time of unbound plasma cefazolin above the MIC ($fT_{>\text{MIC}}$) between opening and closure of the wound is used as the pharmacokinetic/pharmacodynamic (PK/PD) index. However, this is based on the assumption that cefazolin penetration from plasma to the ISF of the subcutaneous tissue is equal to 1⁷, whereas in this study it was found that cefazolin tissue distribution is lower than 1 for morbidly obese patients. This suggests that for morbidly obese patients ISF tissue concentrations rather than unbound plasma concentrations should be considered as the PK/PD index to target for cefazolin efficacy. Monte Carlo simulations allowed evaluation of cefazolin ISF tissue concentrations in large simulated patient populations and indicated that a dose of 2 gram iv cefazolin given prior to incision will be sufficient to prevent wound infections with pathogens for which the MIC is 1 mg/L in a 120 minute surgical procedure. However, when higher MIC values apply (e.g. 2 or 4 mg/L) redosing may be required after 2 hours as the probability of attaining a target of 4 mg/L at 180 minutes post dose has dropped to 0.909 for morbidly obese as opposed to 0.995 for non-obese patients, while for a target of 2 mg/L the probability of target attainment is 0.956 in morbidly obese versus 0.997 in non-obese patients at 240 minutes post dose (Table 3). Alternatively, it is obvious that if surgery is prolonged beyond 4 hours, an extra dose is necessary even when an MIC of 1 mg/L is taken as the reference value.

The design of the current study allowed for a straight forward and extensive comparison of unbound cefazolin concentrations in both plasma and ISF of the subcutaneous adipose tissue in morbidly obese and non-obese patients undergoing laparoscopic gastric surgery. In addition, it allowed for a quantitative analysis of the influence of morbid obesity on cefazolin distribution. Nevertheless, the current study has some limitations. Firstly, this study only included 15 patients, which may limit an accurate estimation of interindividual and residual variability of pharmacokinetic parameters, which in turn may prevent broad conclusions being drawn regarding cefazolin efficacy in morbidly obese patients. Also, extrapolation of this model to patients beyond the body weight ranges of these data should be exercised with caution. However, the data gathered in this study is rather unique both in terms of methods (rich data, semi simultaneous observations in ISF and plasma) and patients, and currently no other evidence about cefazolin efficacy in morbidly obese patients is available. Secondly, the ISF data from one morbidly obese patient was excluded from the pharmacokinetic analysis, because the ISF time-concentration profile of this patient was highly deviating and outlying in comparison with the other morbidly obese patients in this study. This deviation may be explained by the relatively low microdialysis recovery ratio measured for this patient (13.6%, compared to a mean of $28.1\% \pm 7.9$). Thirdly, it should be stated that the model

developed here, slightly overestimates lower subcutaneous cefazolin concentrations in some of the morbidly obese patients. If the model had predicted these lower cefazolin ISF concentrations more accurately, the probability of target attainment results from the Monte Carlo simulation may have been even more disadvantageous for morbidly obese patients. Finally, it is assumed that these potential weaknesses do not explain the lower cefazolin tissue penetration found for morbidly obese patients in this study.

In conclusion, this study showed that cefazolin distribution to the ISF of the subcutaneous adipose tissue is reduced in morbidly obese versus non-obese patient, that cefazolin tissue distribution reduces with increasing body weight and that dose adjustments are required in this patient group.

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TRANSPARENCY DECLARATIONS

None to declare.

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SUPPLEMENTARY MATERIAL TO CHAPTER 4

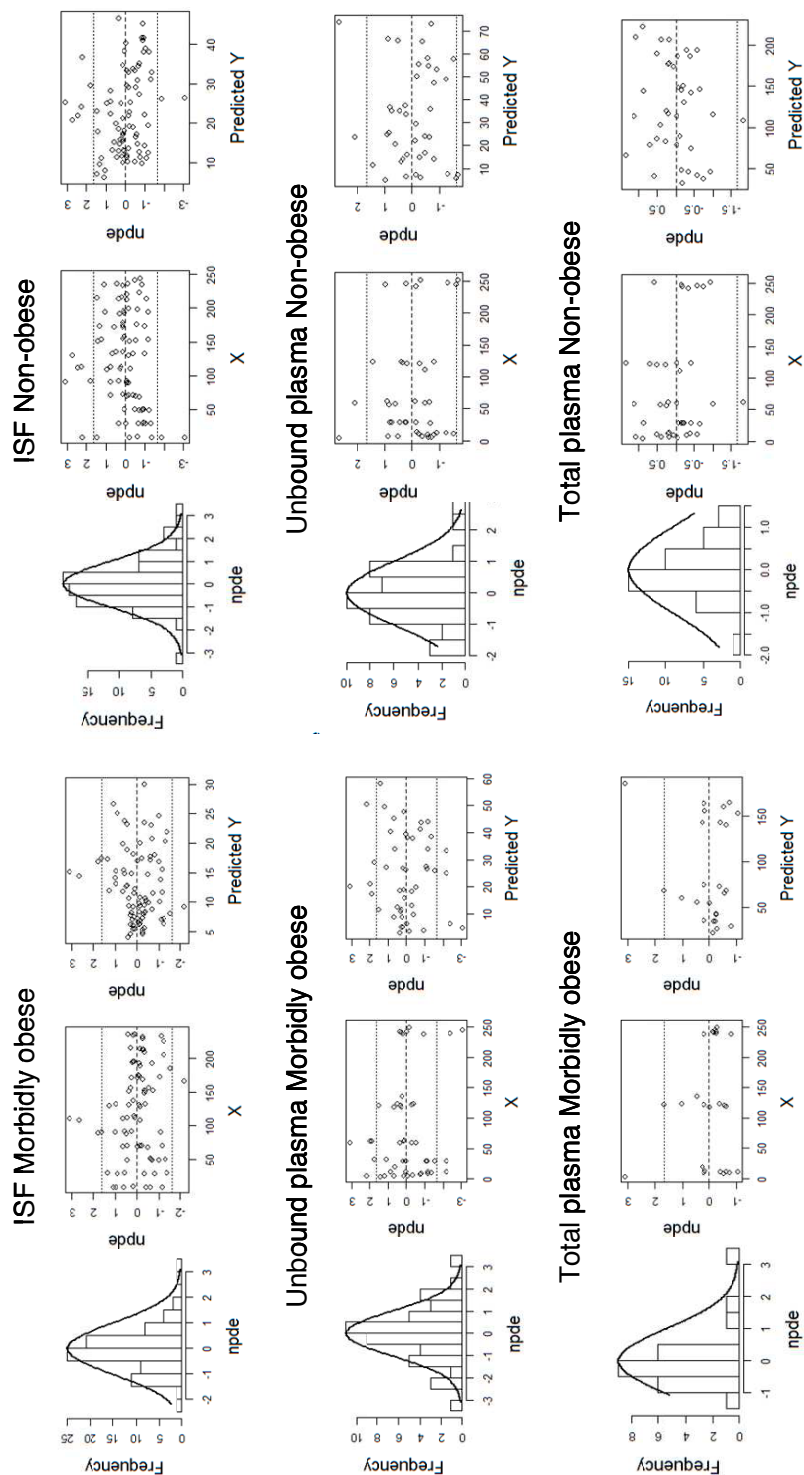
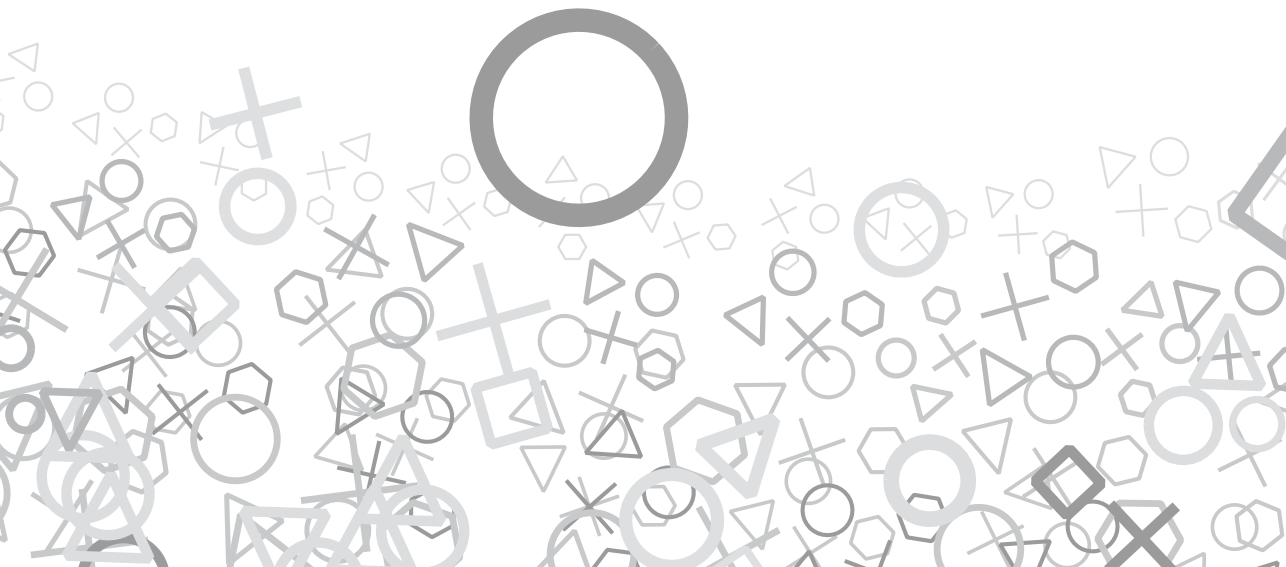


Figure S1 Results of the NPDE analysis. The first graph of each set of three graphs shows the histograms with the distribution of the NPDEs for cefazolin. The solid line depicts a normal distribution and the values below specify the mean and standard deviation of the observed NPDE distribution in the histograms. The distribution of NPDEs in (X) time and against the observed concentrations (predicted Y) are shown in the second and third graph of each set, respectively.



Influence of morbid obesity and weight loss surgery on the pharmacokinetics of CYP3A substrate midazolam



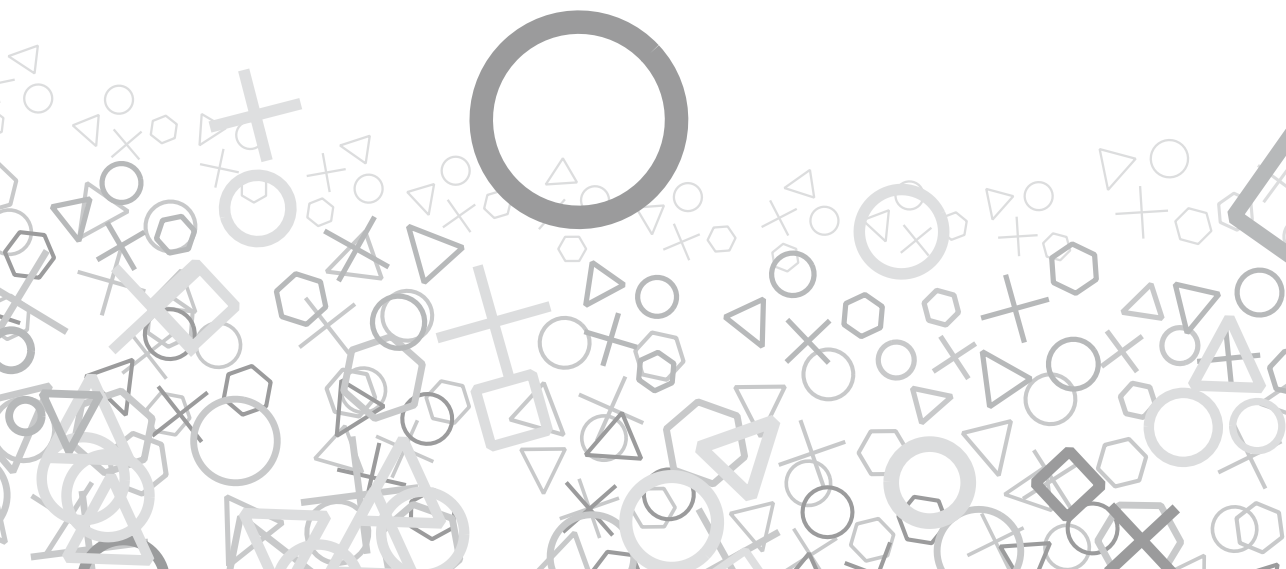


CHAPTER 5

Midazolam pharmacokinetics in morbidly obese patients following semi-simultaneous oral and intravenous administration: a comparison with healthy volunteers

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ABSTRACT

Background

While *in vitro* and animal studies have shown reduced CYP3A activity due to obesity, clinical studies in (morbidly) obese patients are scarce. As CYP3A activity may influence both clearance and oral bioavailability in a distinct manner, in this study the pharmacokinetics of the CYP3A substrate midazolam were evaluated after semi-simultaneous oral and intravenous administration in morbidly obese patients, and compared with healthy volunteers.

Methods

Twenty morbidly obese patients (mean body weight 144 kg (112-186 kg) and mean BMI 47 kg/m² (40-68 kg/m²) participated in the study. All patients received a midazolam 7.5 mg oral and 5 mg intravenous dose (separated by 159 ± 67 minutes) and per patient 22 samples over 11 hours were collected. Data from 12 healthy volunteers were available for a population pharmacokinetic analysis using NONMEM®.

Results

In the three-compartment model in which oral absorption was characterized by a transit absorption model, population mean clearance (relative standard error %) was similar (0.36 (4.4%) L/min), while oral bioavailability was 60% (13.2%) in morbidly obese patients versus 28% (7%) in healthy volunteers ($p < 0.001$). Central and peripheral volumes of distribution increased substantially with body weight (both $p < 0.001$) and absorption rate (transit rate constant) was lower in morbidly obese patients (0.057 (5%) versus 0.130 (14%) min⁻¹, $p < 0.001$).

Conclusions

In morbidly obese patients, systemic clearance of midazolam is unchanged, while oral bioavailability is increased. Given the large increase in volumes of distribution, dose adaptations for intravenous midazolam should be considered. Further research should elucidate the exact physiological changes at intestinal and hepatic level contributing to these findings.

INTRODUCTION

The prevalence of obesity (body mass index, BMI, $>30 \text{ kg/m}^2$) and morbid obesity (BMI $>40 \text{ kg/m}^2$) is increasing rapidly. In 2010, 6.6% (15.5 million) of the U.S adult population was morbidly obese, a 70% increase since 2000¹, while 36% of the U.S. population was obese². In Europe, approximately 20% of the adult population is currently obese³.

In obese mice, studies have shown reduced hepatic Cytochrome P450 3A (CYP3A) protein expression⁴⁻⁵. Similarly, in *in vitro* studies with hepatocytes from human fatty livers, reduced CYP3A expression and activity has been reported⁶⁻⁷ with increasing severity of fatty liver and non-alcoholic steatohepatitis (NASH), which are both highly associated with (morbid) obesity⁸⁻⁹. However, these measurements concern absolute values and were not normalized for the weight of the whole liver and/or the body weight of the mouse. CYP3A is an important enzyme system that is responsible for the primary metabolism of 25% of all clinically used drugs¹⁰, including many drugs that are relevant for obese patients, such as statins, cardiovascular drugs, antipsychotics and oncolytic drugs¹¹. In obese as compared to non-obese subjects, it was shown that hepatic and intestinal CYP3A protein expression decreased with increasing BMI¹² and that oral clearance (CL/F) of CYP3A substrates such as triazolam, carbamazepine and taranabant was lower¹³⁻¹⁴, even though a similar systemic clearance (CL) was found in obese individuals for midazolam¹⁵. An explanation for the reduction in CYP3A activity upon obesity could be an increased state of inflammation, caused by infiltration of macrophages and adipocytes into the adipose tissue excreting inflammation markers and adipokines, such as interleukin (IL)-6 and tumor necrosis factor (TNF)- α ¹⁶⁻¹⁸. Both *in vitro* and animal studies have shown that inflammation factors such as IL-6 may decrease CYP3A expression, resulting in down regulation of CYP3A mediated metabolism¹⁹⁻²³. Finally, reduced CYP3A activity due to inflammation has also been shown in critically ill patients²⁴⁻²⁵.

Midazolam which is considered a specific marker of CYP3A activity because it is primarily metabolized by CYP3A²⁶, is a widely applied oral or intravenous drug for sleeping disorders, (pre)anesthesia, sedation for scopic interventions and in the intensive care unit. As CYP3A is located in both the liver and the intestines, the activity of the CYP3A enzyme is an important determinant of midazolam systemic clearance (CL) and oral bioavailability (F)²⁷⁻²⁸. In view of the ever-increasing body weights of morbidly obese patients, in this pharmacokinetic study we evaluate the influence of morbid obesity on CYP3A-mediated systemic clearance and oral bioavailability of midazolam when studied after semi-simultaneous oral and intravenous administration, allowing these parameters to be characterized in a distinct manner. For the analysis, midazolam data from a healthy volunteer study with the same study design were also available. The results of this study are used to illustrate the consequences for dosing of oral and intravenous midazolam in morbidly obese patients.

METHODS

Study design and patients

This prospective observational study in morbidly obese patients (NTC01519726 and EudraCT 2011-003293-93) was approved by the local human research and ethics committee of the Sint Antonius Ziekenhuis (VCMO, NL35861.100.11) and conducted in accordance with the principles of the Declaration of Helsinki and the Medical Research Involving Human Subjects Act (WMO) of the Netherlands. Before participation, all patients gave written informed consent.

Adult morbidly obese patients ($\text{BMI} > 40 \text{ kg/m}^2$) undergoing laparoscopic gastric bypass or sleeve surgery were eligible for inclusion in the study. Patients were excluded if they used CYP3A inducing or inhibiting medication according to Cytochrome P450 Drug Interaction Table ¹¹, used products containing grapefruit, wild grape, banpeiyu, pomegranate, star fruit or black berry within 2 weeks before the study, were pregnant or breastfeeding, or suffered from renal insufficiency (estimated glomerular filtration rate (Modification of Diet in renal Disease, MDRD4, $< 60 \text{ mL/min}$)).

Study procedure

Twenty morbidly obese patients were studied on the day of laparoscopic bariatric surgery after an overnight fast. Midazolam was administered in a semi-simultaneous manner. Approximately 2.5 hours before induction of anesthesia, patients received midazolam 7.5 mg orally as a tablet (Dormicum®, Roche). At the induction of anesthesia (159 ± 67 minutes after the oral dose), 5 mg of intravenous midazolam (Midazolam, Actavis 5mg/ml) was administered. Blood samples were collected at $T = 0, 5, 15, 30, 45, 55, 65, 75, 90, 120, 150$ minutes after the oral dose and $T = 5, 15, 30, 30, 90, 120, 150, 180, 210, 270, 330, 390, 510$ minutes after the intravenous dose. Blood samples were collected in lithium heparin tubes and centrifuged at 3,000 rpm for 10 minutes at 4° C. Plasma was separated and immediately stored at -80 ° C until analysis.

Blood samples to measure markers of liver function (aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), bilirubin, gamma-glutamyltransferase (gamma GT), and albumin) in morbidly obese patients were collected before the oral midazolam dose was administered.

Control group

Data from 12 healthy volunteers receiving midazolam in an identical semi-simultaneous dosing design were available for analysis (EudraCT 2009-010331-40). Subjects had to fast from 2 hours before drug administration and received a 2 mg oral midazolam solution (Midazolam, Synthon) at 10:00 am. After 150 minutes they received a 1 mg intravenous midazolam dose (Midazolam, Synthon) and blood samples were collected at $T = 15, 30,$

45, 60, 65, 70, 75, 80, 90, 120, 148, 155, 165, 180, 210, 240, 270, 330, 390 minutes after oral dose.

Drug assay

In the plasma samples from morbidly obese patients, midazolam was analyzed using a MassTox® TDM series A BASIC-Kit for ultra performance liquid chromatography (UPLC)-tandem mass spectrometry (MS/MS) from Chromsystems Instruments & Chemicals GmbH (Gräfelfing/München, Germany), a commercially available kit including mobile phases, dilution buffers and extraction buffer. For sample preparation, 25 µl of MassTox® BASIC-Kit A extraction buffer was added to 50 µl of the sample. After short vortex mixing, this mixture was let to incubate for 2 minutes. Then 250 µl MassTox® BASIC-Kit A internal standard mix was added and the mixture was vortexed for 30 seconds and centrifuged at 15,000 g for 5 minutes. Ten µl of the supernatant was injected onto the ULPC column (Chromsystems MasterColumn) without a precolumn using a Waters Acquity UPLC system connected to a Waters TQD mass spectrometer with electrospray ionization. The column was kept at 25° C. Eluent was used at a flow rate of 0.6 ml/min. Intra-assay and inter-assay coefficients of variation were 4.7% and 3.3%, respectively. Midazolam recovery was 90%. The lower limit of quantification (LLOQ) was 0.8 ng/ml.

In the plasma samples from healthy volunteers, midazolam was measured using a validated liquid chromatographic-tandem mass spectrometric (LC-MS/MS) assay. Briefly, 500 µl acetonitrile containing midazolam-D4 (4µg/L) was added to 200 µl serum. After 3 min vortex mixing and 5 min centrifugation at ambient temperature the supernatant was collected and transferred into an autosampler vial. Next, 10 µl was injected on an Atlantis T3 C18 3µm column (2.1 x 50 mm; Waters), protected with a guard column (ODS; 4 x 3 mm), which was kept at 30 °C. Gradient elution was performed with a mobile phase consisting of 0.1% v/v aqueous formic acid and 0.1% v/v formic acid in acetonitrile at a flow rate of 0.3 ml/min. The effluent was monitored with a Micromass Quattro Micro triple-quadrupole mass spectrometric detector (Waters). The detector was operated in the positive electrospray ionization mode and configured in the multiple reaction monitoring (MRM) mode. Within-day and between-day inaccuracy and imprecision were less than 5%. The LLOQ was 0.3 ng/mL.

Population pharmacokinetic analysis and internal validation

Population pharmacokinetic modeling was performed on all data by means of nonlinear mixed effects modelling using NONMEM® (version 7.2; GloboMax LLC, Hanover, MD, USA)²⁹. Pirana (2.7.1; Pirana Software & Consulting BV) and R (2.15) were used to visualize the data. All midazolam plasma concentration values that were received from the laboratory were inserted in the datafile, even if these were below the limit of quantifica-

tion (LOQ). Of the 434 samples from morbidly obese patients, 42 were below the LOQ. For healthy volunteers, no data was below the LOQ.

Discrimination between different 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 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) 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 model. The internal validity of the population pharmacokinetic model was assessed by the bootstrap re-sampling method using 500 replicates and normalized prediction distribution errors (NPDE) using 1,000 simulation of each dataset³⁰. Parameters obtained with the bootstrap replicates were compared with the estimates obtained from the original dataset. NPDE plots were checked for normal distribution characteristics and trends in the data errors³⁰.

For the structural model, one-, two, and three-compartment models with an oral dosing compartment were tested. To describe the midazolam oral absorption phase, zero order and first order absorption models were tested, in addition to a lag time model and a transit absorption model³¹. For the transit absorption model, a varying number of transit compartments was tested. As the transit rate (K_{tr}) was set equal to the absorption rate (K_a), the mean transit time (MTT) can be calculated from K_{tr} with $(n+1)/K_{tr}$ in which n is the number of transit compartments³².

For the statistical model, the individual parameter estimate (empirical Bayes estimate or post hoc value) of the i th individual was modelled according to (equation 1):

$$\theta_i = \theta_{mean} \times e^{\eta_i} \quad (\text{Eq. 1})$$

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 and a combined proportional and additive model was tested for each of the data sets. The j th observed midazolam concentration of the i th healthy volunteer (Y_{ij}) is described by Eq. 2, while the j th observed midazolam concentration of the i th morbidly obese patient (Y_{ij}) is described by Eq. 3

$$Y_{ij} = C_{pred,ij} \times (1 + \epsilon_{ij}) \quad (\text{Eq. 2})$$

$$Y_{ij} = C_{pred,ij} \times (1 + \epsilon_{ij}) + \epsilon_{ij} \quad (\text{Eq. 3})$$

where $C_{pred,ij}$ is the population 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 .

Covariate analysis

Covariates were plotted independently against the individual *post hoc* values and eta estimates of pharmacokinetic parameters to visualize potential relations. The following covariates were tested: total body weight (TBW), BMI, lean body weight (LBW) ³³, sex, morbid obesity and age. All covariates except for sex and morbid obesity were tested using linear and allometric equations (Eqs. 4 and 5):

$$P_i = P_p \times (1 + W \times (COV_i - COV_{median})) \quad (\text{Eq. 4})$$

$$P_i = P_p \times \left(\frac{COV}{COV_{median}} \right)^x \quad (\text{Eq. 5})$$

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; W represents a correlation factor between the population pharmacokinetic parameters and the change in covariate value; and X represents the exponential scaling factor for a power function. The binary covariates 'sex' and 'morbid obesity' were tested using Eq. 6:

$$P_i = P_p \times Z^{COV} \quad (\text{Eq. 6})$$

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 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. When more than one significant covariate for the simple model was found, the covariate-adjusted model with the largest decrease in the OFV was chosen as a basis to sequentially explore the influence of additional covariates with the use of the same criteria. Finally, after forward inclusion ($p < 0.05$), a backward exclusion procedure was applied to justify the inclusion of a covariate ($p < 0.01$). The choice of the covariate model was further evaluated as discussed in the *Population pharmacokinetic analysis and internal validation* section.

Model simulations

Using NONMEM® 7.2, the final population pharmacokinetic model was used to simulate the concentration-time profiles of four typical patients from the dataset, including one healthy volunteer of 76 kg and three morbidly obese patients of 112, 145 and 186 kg. The 76 kg and 145 kg dose simulations represent the median body weight of the healthy volunteer and morbidly obese patient group, respectively. In addition, the 112 kg and 186 kg dose simulations represent the extremes of the body weight range of the morbidly obese patient group (see Table 1).

RESULTS

Patients and data

Twenty morbidly obese patients participated in this study and a mean of 22 ± 3 samples per patient were available for analysis. In addition, data from 12 healthy volunteers with 19 midazolam concentrations per subject were used as a control group in this analysis.

Liver function markers in morbidly obese subjects were all within three times the upper limit of normal, with the vast majority being within two times the upper limit of normal of the different markers. The demographics of all subjects are summarized in Table 1.

Table 1 Characteristics of 20 morbidly obese patients and 12 healthy volunteers

	Morbidly obese patients (n=20)	Range	Healthy volunteers (n=12)	Range	P-value
Female/Male	12/8		0/12		
Age (years)	43.6 ± 7.6	26 - 57	22.0 ± 3.1	18 - 27	P < 0.001
Body weight (kg)	144.4 ± 21.7	112 - 186	76.0 ± 8.7	63 - 93	P < 0.001
Lean body weight (kg) ³³	71.5 ± 11.9	53 - 95	61.2 ± 5.0	53 - 70	P < 0.005
BMI (kg/m ²)	47.1 ± 6.5	40 - 68	22.3 ± 2.4	19 - 26	P < 0.001
Number of samples per patient	21.7 ± 2.7	13-24	19 ± 0.0	19 - 19	P < 0.001
Samples below the limit of quantification (%)	9.7	-	0	-	-

Values are expressed as mean ± standard deviation (range) unless specified otherwise

Population pharmacokinetic model and validation

A three-compartment model with two equalized peripheral volumes of distribution best fitted the data. This model showed an improved fit over a two-compartment model, while a full three compartment model could not be estimated with adequate precision. The pharmacokinetic model was parameterized in terms of the oral K_a , oral bioavailability (F), volume of distribution of the central compartment (V1), two equalized volumes of distribution of the peripheral compartments (V2 and V3), inter-compartmental clear-

ances from the central compartment to each peripheral compartment (Q and Q2), and clearance from the central compartment (CL). Midazolam oral absorption described by three transit absorption compartments proved superior over the other oral absorption models (zero and/or first order absorption models or a lag time model) (Figure 1). An omega block was implemented to account for the correlation between the central and peripheral volume of distribution. Table 2 shows the parameter estimates of the simple model without covariates.

In the covariate analysis, a significant influence of TBW or 'morbid obesity' was found on four different parameters, which is visualized in Figure 2 where the post hoc parameters of the simple model without covariates are given. It was found that the peripheral volumes of distribution increased in a non-linear manner with TBW ($p < 0.001$, $-24 \Delta\text{OFV}$), and that the central volume of distribution showed a linear increase with body weight ($p < 0.001$, $-17 \Delta\text{OFV}$). For oral bioavailability and absorption rate (or Ktr), 'morbid obesity' was a significant covariate and significantly improved the model ($p < 0.001$, $-22 \Delta\text{OFV}$ and $p < 0.001$, $-20 \Delta\text{OFV}$, respectively). For clearance, there was a trend towards a positive influence of LBW but not for TBW; however, the statistical significance was insufficient for inclusion of LBW in the final covariate model ($p < 0.05$, $-4 \Delta\text{OFV}$), the estimated correlation factor was not estimated with adequate precision and the eta for clearance value

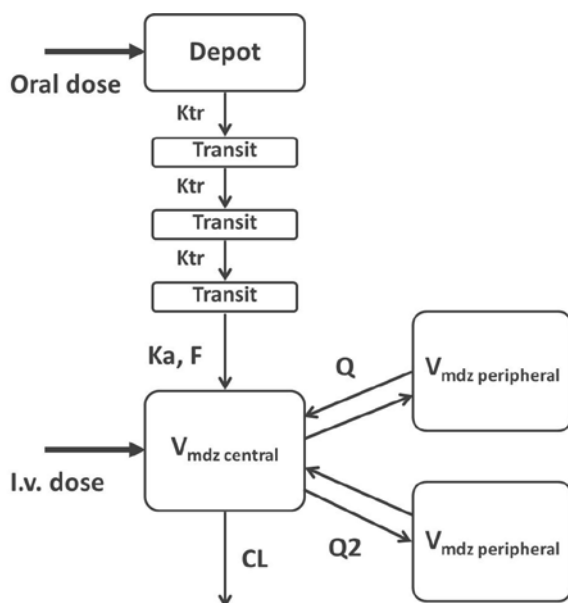


Figure 1 Schematic illustration of the population pharmacokinetic model of oral and intravenous administered midazolam. CL= clearance, F= oral bioavailability, K_a = oral absorption rate, K_{tr} = transit rate constant, MDZ= midazolam, Q= intercompartmental clearance to first peripheral volume, Q2= intercompartmental clearance to second peripheral volume, V= volume of distribution.

was not reduced. Eta distributions for clearance versus body weight and LBW of the simple and final covariate model are included in the Electronic Supplementary Material (ESM 1, see end of this Chapter). After inclusion of the covariates in the model, the trends in eta value of the parameter and the covariate had disappeared and no residual trends were observed (Electronic Supplementary Material, ESM 2). All parameter estimates of the final model are shown in Table 2. Figure 3 demonstrates the goodness-of-fit plots of the final covariate model. The plots show no remaining bias for predicted midazolam concentrations in morbidly obese patients or healthy volunteers. The final model was internally validated by means of 500 bootstrap runs (Table 2), which were successful

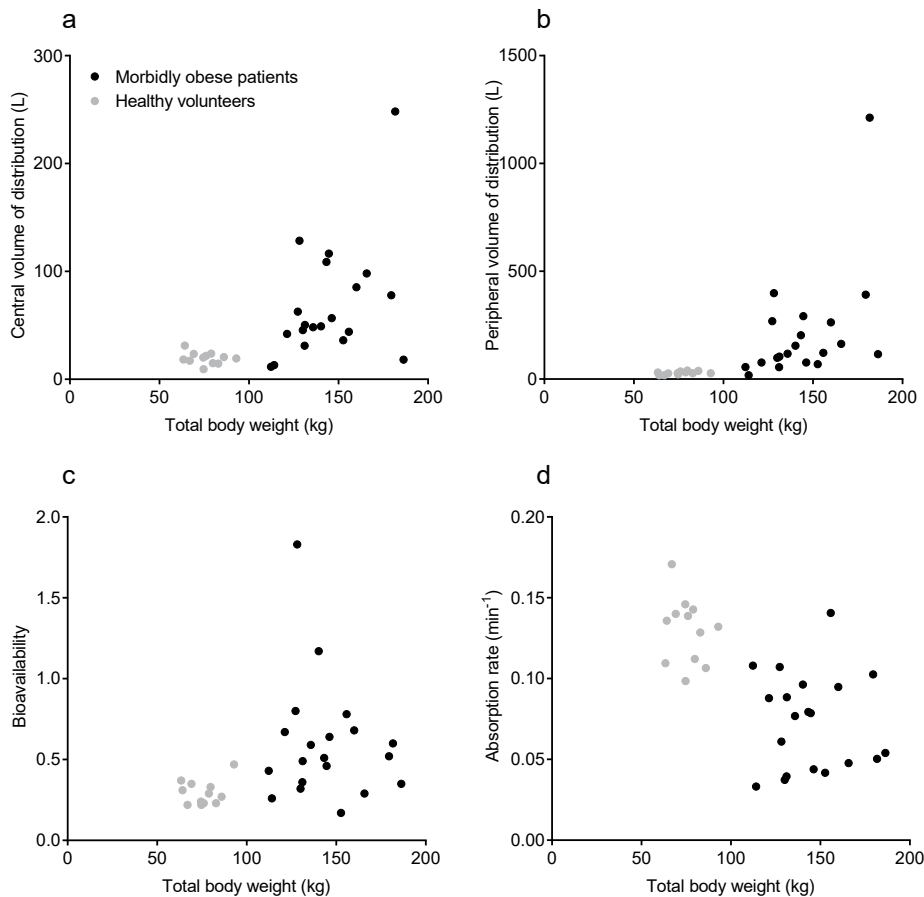


Figure 2 Post hoc parameter estimates of morbidly obese individuals (n=20, black dots) and healthy individuals (n=12, grey dots) from the simple pharmacokinetic model versus total body weight, including central volume of distribution versus total body weight (a), peripheral volume of distribution versus total body weight (b), oral bioavailability versus total body weight (c), and oral absorption rate versus total body weight (d).

Table 2 Population pharmacokinetic parameters of the simple and final pharmacokinetic model for midazolam in 20 morbidly obese patients and 12 healthy volunteers and results from a bootstrap analysis of the final model (479/500 resamples successful).

Parameter	Simple model (RSE%)	Final model (RSE%)	Bootstrap (SE)
<i>Fixed effects</i>			
CL (L/min)	0.36 (4.9)	0.359 (4.4)	0.358 (0.016)
F	0.414 (12.8)	-	
F morbidly obese	-	0.603 (13.2)	0.603 (0.081)
F healthy volunteers	-	0.284 (7.0)	0.286 (0.020)
K _a (min ⁻¹) = K _{tr}	0.086 (3.4) ^a	-	
K _a = K _{tr} morbidly obese	-	0.057 (13.6) ^b	0.058 (0.059)
K _a = K _{tr} healthy volunteers	-	0.13 (5.1) ^b	0.130 (0.006)
V _{central} (L)	36.4 (7.8)	-	
V _{central} = V _{127 kg} * (1 + Z * (TBW - 127))			
V _{central, 127 kg}	-	44.1 (16.1)	43.6 (6.9)
Z	-	0.0105 (15.8)	0.0102 (0.002)
V _{midazolam peripheral} (L)	76.6 (8.4)	-	
V _{peripheral} = V _{127 kg} * (TBW/127) ^W			
V _{peripheral, 127 kg}	-	139 (15.2)	138.7 (22.9)
W	-	3.06 (8.2)	3.07 (0.28)
Q (L/min)	1.31 (12.8)	1.33 (11.8)	1.33 (0.143)
Q2 (L/min)	0.153 (12.1)	0.15 (14.6)	0.15 (0.023)
<i>Interindividual variability (%)</i>			
CL	19.7% (32.6)	18.1% (30.7)	17.2% (14.6)
F	61.2% (20.8)	26.4% (17.4)	25.4% (14.6)
K _a = K _{tr}	50.7 % (10.9)	41.4% (12.8)	39.9% (18.4)
V _{central}	102.8% (13.2)	55.2% (17.5)	53.5% (30.8)
V _{peripheral}	152.3% (13.9)	34.4% (26.5)	33.6% (23.7)
Correlation between eta V _{central} and V _{peripheral}	0.783 (50.0)	0.12 (24.5)	0.10 (0.058)
<i>Residual variability (%)</i>			
Proportional error Healthy volunteers	10.0% (21.5)	10.0% (21.3)	9.9% (4.4)
Proportional error Morbidly obese patients	31.0% (17.1)	46.7% (11.6)	46.6% (15.6)
Additive error morbidly obese patients	3.1 (37.3)	-	-
OFV	4077	4003	3982 (145)

CL= systemic clearance of midazolam, F= oral bio-availability, K_a= oral absorption rate, K_{tr}= transit compartment rate, V= volume of distribution, Q= inter-compartmental clearance of midazolam between central and first peripheral compartment, Q2= inter-compartmental clearance of midazolam between central and second peripheral compartment, OFV= Objective Function Value, RSE= relative standard error, SE= standard error, TBW= total body weight, V= volume of distribution

^a Mean transit time is 46.5 minutes.

^b Mean transit times are 70.2 and 30.8 minutes, respectively.

in 96% of the runs and confirmed the parameter values. Finally, an NPDE analysis was performed showing a normal distribution of errors without trends, except for the initial midazolam concentrations in morbidly obese patients, which were below the LOQ (see Electronic Supplementary Material , ESM 3). The NONMEM® control file of the final model is provided as online resource (ESM 4).

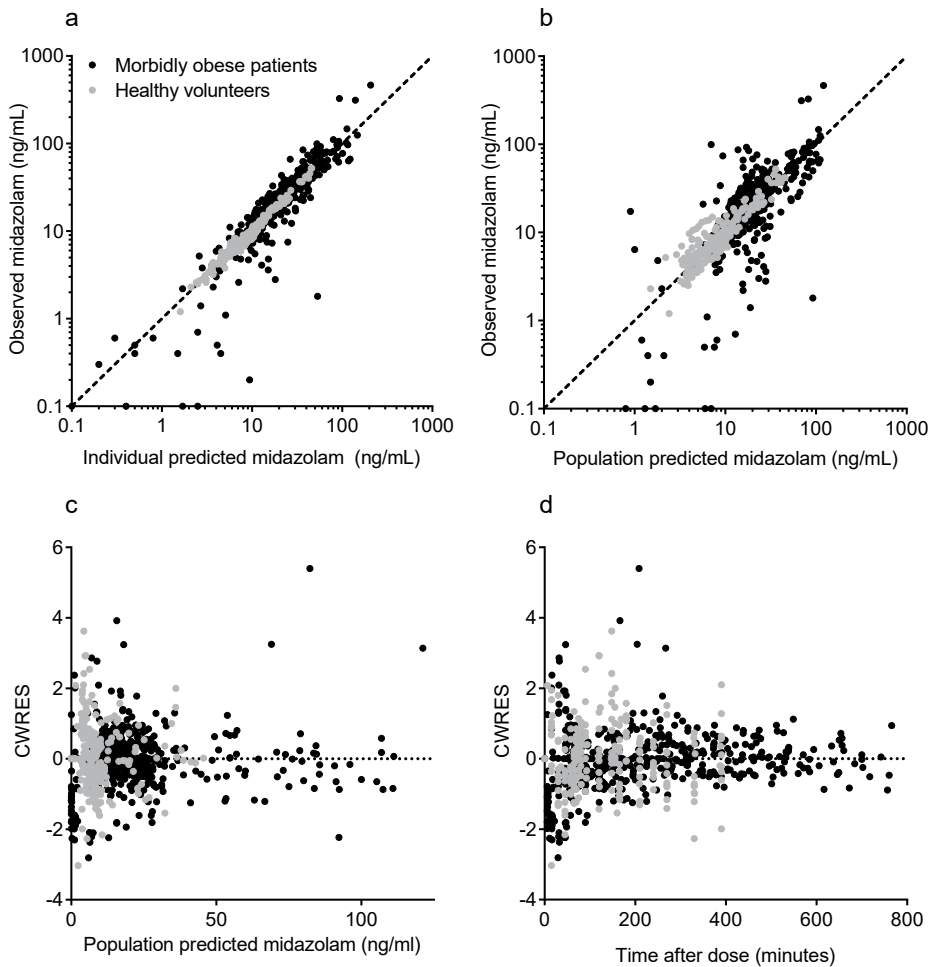


Figure 3 Observed versus individual predicted midazolam concentrations (a), observed versus population predicted midazolam concentrations (b), conditional weighted residuals (CWRES) versus population predicted midazolam concentrations (c) and conditional weighted residuals (CWRES) versus time of the final model for 20 morbidly obese patients (black dots) and 12 healthy volunteers (grey dots). The dashed line represents the line of identity ($x=y$).

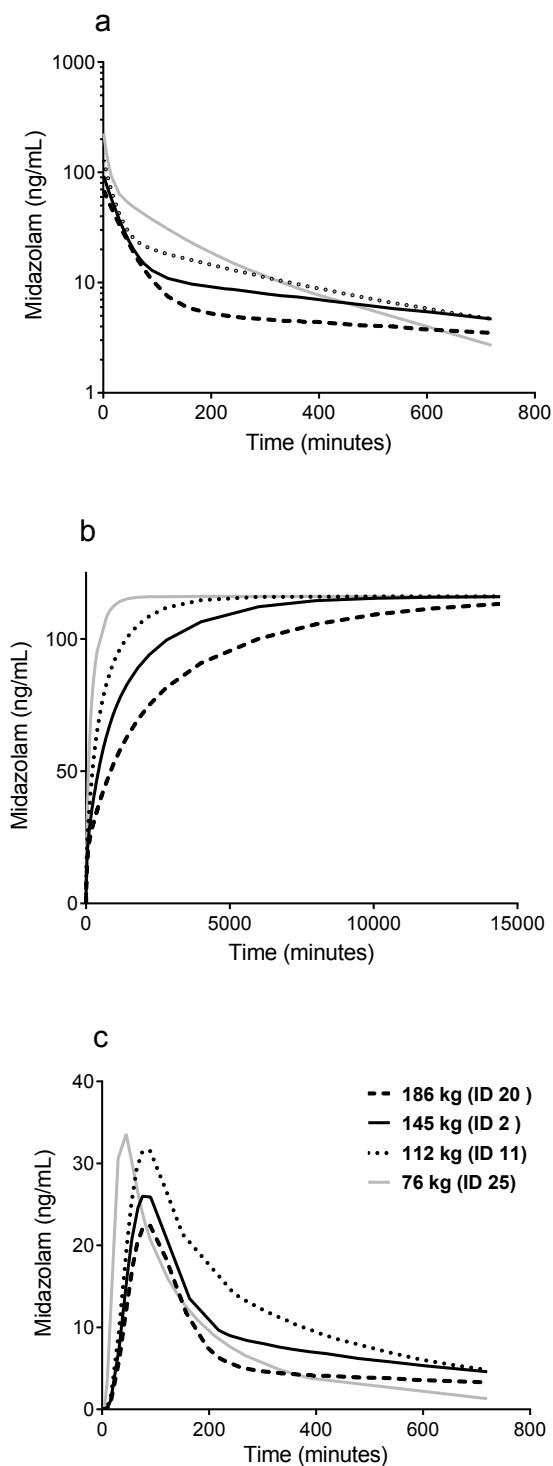


Figure 4 Population predicted midazolam concentrations over time in three typical morbidly obese patients (112, 145 and 186 kg) and one healthy volunteer (76 kg) after a 5 mg intravenous bolus dose (logarithmic scale) (a), a 2.5 mg/h continuous infusion (linear scale) (b) and a 7.5 mg oral dose (linear scale) (c).

Simulations

Figure 4 shows population predicted midazolam concentrations after a 5 mg intravenous bolus dose, a 2.5 mg/h continuous infusion for a duration of 10 days (14,400 minutes) and a 7.5 mg oral dose in 4 typical subjects from the dataset (i.e. 76 kg, 112, 145 and 186 kg). The plot shows that for the intravenous bolus dose, midazolam (peak) concentrations ($C_{\max}$) are lower in morbidly obese patients (Figure 4a), which may be the result of the higher central volume of distribution in morbidly obese patients. In addition, the plot illustrates the longer midazolam half-life ($t_{1/2}$) in morbidly obese patients, which can be attributed to the increase in volumes of distribution with body weight, while clearance is the same in all patients. The continuous intravenous infusion simulation (Figure 4b) shows that with increasing body weight the midazolam steady-state concentrations are reached at a later time point. In a 76 kg healthy volunteer, steady-state is expected to be reached after 24 hours, while this is 170 hours in a 145 kg morbidly obese patient and >240 hours in a 186 kg morbidly obese patient (Figure 4b). Finally, the oral midazolam dose simulations (Figure 4c) show that the time to reach the $C_{\max}$ is later (31 minutes) in morbidly obese patients than in healthy volunteers, while the $C_{\max}$ is slightly lower in morbidly obese patients.

DISCUSSION

As there is only limited information on the influence of morbid obesity on CYP3A-mediated clearance of drugs in patients, this study aimed to evaluate the pharmacokinetics of CYP3A substrate midazolam in morbidly obese patients following oral and intravenous midazolam administration. As clearance after oral dosing is dependent on oral bioavailability, which may be influenced by CYP3A enzyme activity in the intestines, this semi-simultaneous design allows for an estimation of both systemic clearance and oral bioavailability in a distinct manner. An available dataset of midazolam concentrations collected on the basis of an equivalent study design in healthy volunteers allowed for a head-to-head comparison between morbidly obese patients and non-obese healthy subjects. The results from this study show that midazolam clearance was similar in morbidly obese patients and healthy volunteers, oral bioavailability was substantially higher (60% instead of 28%), oral absorption rate was reduced and that the central and peripheral volumes of distribution increased substantially with body weight. Particularly for intravenous dosing, the net results of all these changes should be considered when administering midazolam to morbidly obese patients.

In this study, we could not identify an influence of morbid obesity on the systemic clearance (CL) of midazolam, even though a wide range in body weights was included in this study. We did find a trend of increasing midazolam clearance with lean body

weight; however, this trend was not strong enough for inclusion in the final covariate model. Possibly, the patient numbers in this analysis ($n=12 + n=20$) are insufficient to adequately detect a small increase in clearance with LBW. While these results indicate a lack of change in absolute hepatic CYP3A mediated metabolism of midazolam in morbidly obese individuals, the results are in contrast with our expectations of a lower midazolam clearance in morbidly obese patients which was based on reports in *in vitro* and animal studies⁴⁻⁷ and on oral clearance of CYP3A substrates in studies in obese subjects¹³⁻¹⁴. Assuming that indeed the relative CYP3A activity per unit of liver is reduced in morbidly obese patients¹², we hypothesize that this effect may be counteracted by a higher liver volume³⁴, resulting in a similar absolute hepatic CYP3A metabolising capacity in both groups. In agreement with this hypothesis, also Greenblatt *et al.* found no significant difference in absolute systemic clearance of midazolam between normal weight (66 ± 2 kg) and obese subjects (117 ± 8 kg) (0.53 ± 0.04 vs. 0.47 ± 0.04 L/min, respectively)¹⁵. However for a study with triazolam, another benzodiazepine CYP3A substrate, a lower oral clearance (CL/F) was found for obese patients³⁵. Lower CL/F values were also found for obese patients for the CYP3A substrates taranabant and carbamazepine³⁶⁻³⁷. Based on the results found in the current midazolam study upon both oral and intravenous administration, it may be hypothesized that these lower oral clearance (CL/F) values³⁵⁻³⁷ are due to an increase in oral bioavailability (F) instead of a decrease in systemic clearance (CL).

Oral bioavailability was found to be higher in morbidly obese patients compared to healthy volunteers (0.60 (13.2%) versus 0.28 (7.0%)). In contrast, Greenblatt *et al.* found similar values of oral midazolam bioavailability in obese (0.42 ± 0.04) and normal weight patients (0.40 ± 0.03) ($p>0.05$). This disagreement in results may be explained by the higher body weights of the morbidly obese subjects in our study versus the study of Greenblatt *et al.* (mean of 144 ± 22 versus 117 ± 8 kg). In addition, the concentration-time profiles after oral and intravenous midazolam of a non-obese and an obese subject shown in their publication, may also point at a higher bioavailability in the obese patient¹⁵. We anticipate that the increase in oral bioavailability in morbidly obese patients found in our study may be due to reduced CYP3A metabolizing activity in the intestines. Ulvestad *et al.* found in a study with 19 obese individuals (median BMI 45 (34-59) kg/m²) that CYP3A4 protein expression in the small intestine and liver is lower with increasing BMI. Another possible cause of increased bioavailability is an increase in splanchnic blood flow, which has been reported before in morbidly obese patients³⁸. An increase in villous blood flow in the gut wall will cause an increase in substrate transport and thus carry the substrate away from the intestinal CYP3A metabolizing enzymes³⁹⁻⁴⁰. Moreover, increased intestinal permeability may be responsible for increased midazolam bioavailability as obese patients showed increased paracellular absorption measured with lactulose and chromium (Cr)-EDTA, which may possibly be due to reduced tight

junction function⁴¹⁻⁴². A question would be whether the observed difference in absorption rate (K_a) between the morbidly obese patients and healthy volunteers, which can be attributed to a difference in formulation (tablet versus oral solution), may have contributed to the reported difference in oral bioavailability. In our opinion, this difference in formulation is unlikely to influence oral bioavailability, as midazolam is a highly soluble and permeable drug which is expected to be 100% absorbed in the intestines⁴³. Correspondingly, a study in which 6 healthy volunteers received a 10 mg midazolam oral solution, a 10 mg tablet and a 5 mg intravenous bolus dose, showed similar oral bioavailability after both oral dose formulations, 0.35 ± 0.07 versus 0.38 ± 0.12 ($p > 0.6$), indicating no influence of oral formulation on midazolam bioavailability⁴⁴.

To understand the net result of the influence of different degrees of (morbid) obesity on each of the midazolam pharmacokinetic parameters, simulations using the final model were performed to yield midazolam concentration-time profiles for subjects with different body weights. The dose simulations show that the same intravenous bolus dose to all subjects leads to lower initial concentrations in morbidly obese patients due to a substantially higher central and peripheral volume of distribution. This observed increase in volume of distribution is in agreement with the midazolam study of Greenblatt *et al.* in which a substantial increase in total volume of distribution for obese versus normal weight subjects of 311 ± 27 L versus 114 ± 7 L ($p < 0.001$) was also reported¹⁵. Potentially, these results can be explained by an increase in body volume in terms of both well-perfused compartments (organ and blood volume) and adipose tissue with obesity, which is of specific relevance because midazolam is a lipophilic drug^{38, 45}. As such, directly after an intravenous bolus dose, lower midazolam concentrations and associated effects may be expected in morbidly obese patients. In addition, morbidly obese patients show an increased elimination half-life (Figure 4), which can be attributed to the larger volumes of distribution as well, as they allow for significant midazolam disposition from the blood and may lead to prolonged midazolam effects in morbidly obese patients versus healthy volunteers. In contrast, a similar midazolam oral dose will result in only slightly lower initial concentrations in morbidly obese patients versus healthy volunteers because the increased oral bioavailability counteracts the influence of increased central volume of distribution on midazolam peak concentrations (Figure 4). Finally, the increase in the volumes of distribution with body weight also explains the increased duration for morbidly obese patients to reach steady-state concentrations after a continuous intravenous infusion. This phenomenon has been described before for diazepam in obese patients⁴⁶. Therefore a loading dose or a higher initial continuous infusion rate may be considered to reach midazolam steady-state concentrations more rapidly in morbidly obese patients.

There are some limitations to this study. Firstly, the sampling duration after oral administration may have been relatively short. Particularly in morbidly obese patients,

the midazolam peak concentrations after the oral dose occurred at approximately 90 minutes post dose, leaving only a 60 minute time interval to collect data on the concentration decline after oral dose before the intravenous dose was administered. However, in six of the 20 patients this interval was >180 minutes (due to a delay in the surgery schedule), thus providing significant information on the midazolam pharmacokinetics after oral absorption in the morbidly obese patients. Secondly, the healthy volunteer group lacks a late sample post intravenous dose, which may have an effect on the clearance and peripheral volume of distribution estimates of the healthy volunteers and thus obscure the covariate analysis. However, estimated pharmacokinetic parameter values for this group closely match those found in previous midazolam pharmacokinetic studies in healthy volunteers, indicating adequate precision of the pharmacokinetic parameters in healthy volunteers and justifying the results from the covariate analysis. Thirdly, morbidly obese patients underwent surgery during the study, which may influence midazolam clearance and distribution. However, we think that surgery was only of minor influence as only the intravenous dose was administered during surgery and systemic clearance and volume of distribution found in this study were fairly similar to earlier reported values in non-surgery obese patients¹⁵. Finally, the stable isotope method for determining oral bioavailability in a single person on a single occasion may have been preferable over the current semi-simultaneous dosing design. Though, the semi-simultaneous oral-intravenous administration method has proved a reliable and accurate method for estimating oral bioavailability and systemic clearance in a single person, on a single occasion as well⁴⁷⁻⁵⁰. Moreover, the available control data (midazolam concentrations in healthy volunteers) was gathered in a semi-simultaneous design. Lastly, the preparation of the labeled drug and the determination of the labeled drug in the samples is very expensive and labor intensive. For these reasons, we have chosen to apply the semi-simultaneous design.

In conclusion, this study shows that midazolam hepatic clearance was not changed in morbidly obese patients versus healthy volunteers, while oral bioavailability was increased in morbidly obese patients. Midazolam central and peripheral volumes of distribution increased substantially with body weight resulting in lower midazolam concentrations after intravenous bolus administration and in increased duration to reach steady state concentrations after midazolam continuous infusion in morbidly obese patients in comparison to healthy volunteers. Finally, initial midazolam concentrations after an oral dose were similar in morbidly obese patients versus healthy volunteers. Further research should elucidate the exact physiological changes at intestinal and hepatic level contributing to these findings.

ACKNOWLEDGMENTS

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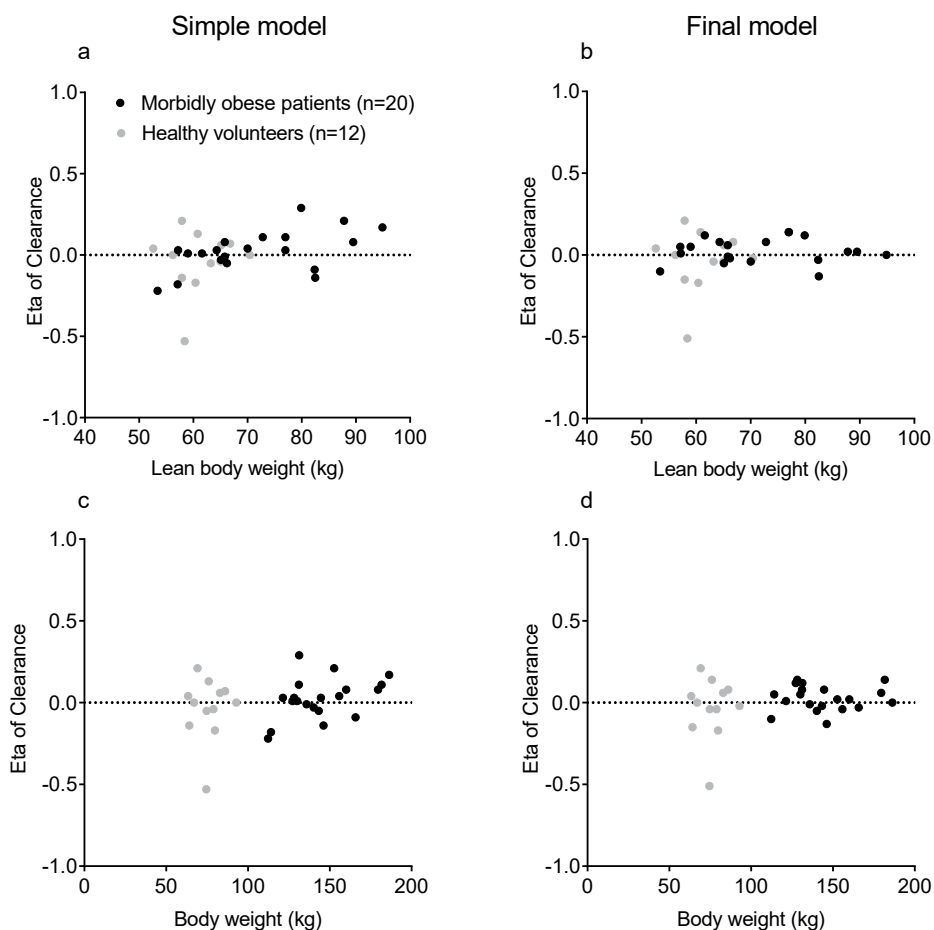
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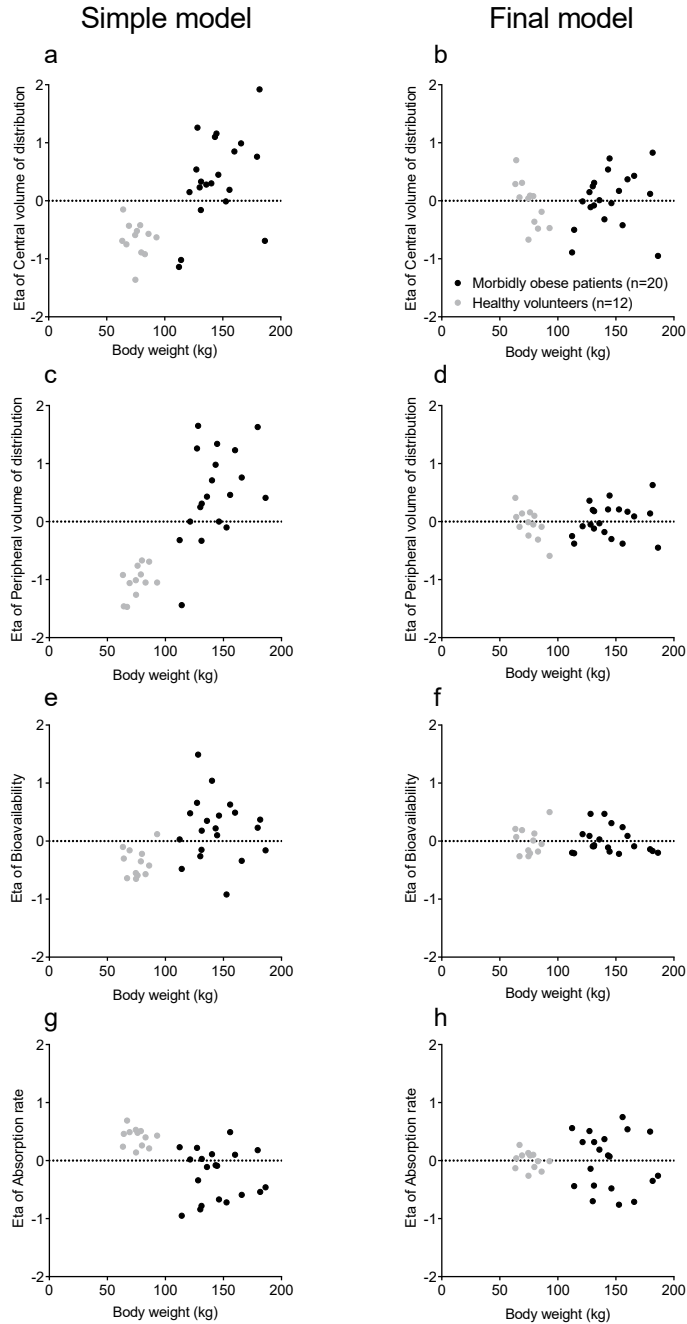
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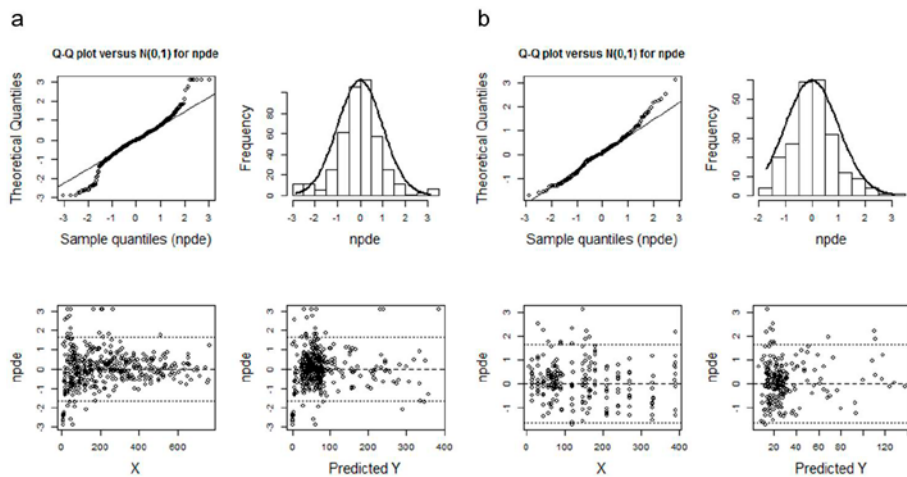
SUPPLEMENTARY MATERIAL TO CHAPTER 5



ESM 1 Eta distributions for clearance for the simple (a and c) and final (b and d) pharmacokinetic model versus lean body weight (a and b) and body weight (c and d) in 20 morbidly obese patients (black dots) and 12 healthy volunteers (grey dots).



ESM 2 Eta distributions for central volume of distribution (a and b), peripheral volume of distribution (c and d), oral bioavailability (e and f), oral absorption rate (K_{tr}) (g and h) in 20 morbidly obese patients (black dots) and 12 healthy volunteers (grey dots) for the simple (a, c, e, g) and final midazolam pharmacokinetic model (b, d, f, h).



ESM 3 Normalized prediction distribution errors (npde) for the midazolam data from 20 morbidly obese patients (a) and from 12 healthy volunteers (b). The X indicates the time after dose and Y indicates the midazolam concentration in nmol/L.

ESM 4 NONMEM control stream of the final pharmacokinetic model

\$PROBLEM Final model #61

\$INPUT ID TIME AMT RATE DROP DV CMT MDV TAD TADI OBES TBW LBW IBW BMI WHR
AGE SEX LOQ IV ROUN

; Parent=run18

\$DATA BOTH.occ1.incl.BLOQ.11.csv

\$SUBROUTINES ADVAN6 TOL=5

\$MODEL

COMP=(PODOSE)

COMP=(CENTRAL)

COMP=(PERIP)

COMP=(PERIP2)

COMP=(TRANSIT1)

COMP=(TRANSIT2)

COMP=(TRANSIT3)

\$PK

TVCL= THETA(1)

CL= TVCL*EXP(ETA(1))

TVF1= THETA(2)*THETA(10)**OBES

F1= TVF1*EXP(ETA(2)) ; oral bioavailability

TVV2= THETA(3)*(1+THETA(8)*(TBW-127))

V2= TVV2*EXP(ETA(5)) ; volume (L)

TVQ= THETA(4)

Q= TVQ*EXP(ETA(3)) ; intercompartmental clearance (L/min)

TVV3= THETA(5)*(TBW/127)**THETA(9)

V3= TVV3*EXP(ETA(6)) ; peripheral volume of distribution (L)

TVKA= THETA(6)*THETA(11)**OBES

KA= TVKA*EXP(ETA(4)) ; oral absorption rate or Ktr (min⁻¹)

KTR= KA

V4= V3 ; 2nd volume periph. compartment (L)

Q4= THETA(7) ; clearance to 2nd periph. compartment (L/min)

;

S2=V2

```

S3=V3
;
K15=KA
K56=KTR
K67=KTR
K72=KTR
K20=CL/V2
K23=Q/V2
K32=Q/V3
K24=Q4/V2
K42=Q4/V4
$DES
DADT(1)= -K15*A(1)
DADT(2)= KTR*A(7) -K23*A(2) +K32*A(3) -K24*A(2) +K42*A(4) -K20*A(2)
DADT(3)= K23*A(2) -K32*A(3)
DADT(4)= K24*A(2) -K42*A(4)
DADT(5)= K15*A(1) -KTR*A(5)
DADT(6)= KTR*A(5) -KTR*A(6)
DADT(7)= KTR*A(6) -KTR*A(7)
$ERROR
COM1=0
IF (OBES.EQ.0) COM1=1
COM2=0
IF (OBES.EQ.1) COM2=1
;
IPRED=F ; individual prediction
;
Y1=IPRED*(1+ERR(1)) ; healthy volunteers
Y2=IPRED*(1+ERR(2)) ; morbidly obese patients
;
Y=Y1*COM1+Y2*COM2
;
IRES=DV-IPRED ; individual residual
DEL=0 ; if F=0, than see next lines
IF(IPRED.EQ.0)DEL=1
IWRES=(1-DEL)*IRES/(IPRED+DEL)
$THETA
(0, 0.2) ;CL (L/min)
(0, 0.3) ;F1 healthy volunteers

```



```

(0, 30) ;V2 (L)
(0, 0.9) ;Q (L/min)
(0, 100) ;V3 (L)
(0, 0.1) ;KA healthy volunteers
(0, 0.1) ;Q4
(0, 0.003) ;TBW V2
(0, 2) ;TBW V3
(0, 2) ;fraction F1 morbidly obese
(0, 0.5) ;fraction KA morbidly obese
;
$OMEGA
0.03 ;CL
0.1 ;F1
0 FIX ;Q
0.2 ;KA
$OMEGA BLOCK(2)
0.3 ;V2 (central)
0.05 0.1 ;V3 (peripheral)
;
$SIGMA
0.005 ; healthy volunteers
0.1 ; morbidly obese
$EST SIGDIG=3 MAXEVAL=9999 PRINT=5 NOABORT METHOD=1 INTERACTION POSTHOC
$COV COMP
$TABLE ID TIME TAD TADI IPRED IWRES CWRES CMT ROUN OBES IV NOPRINT ONEHEADER
FILE=sdtab61
$TABLE ID TAD CMT TADI ROUN OBES IV CL V2 Q V3 Q4 KA F1 V4 TBW LBW IBW AGE
BMI WHR ETA(1) ETA(2) ETA(3) ETA(4) ETA(5) ETA(6) NOPRINT NOAPPEND ONEHEADER
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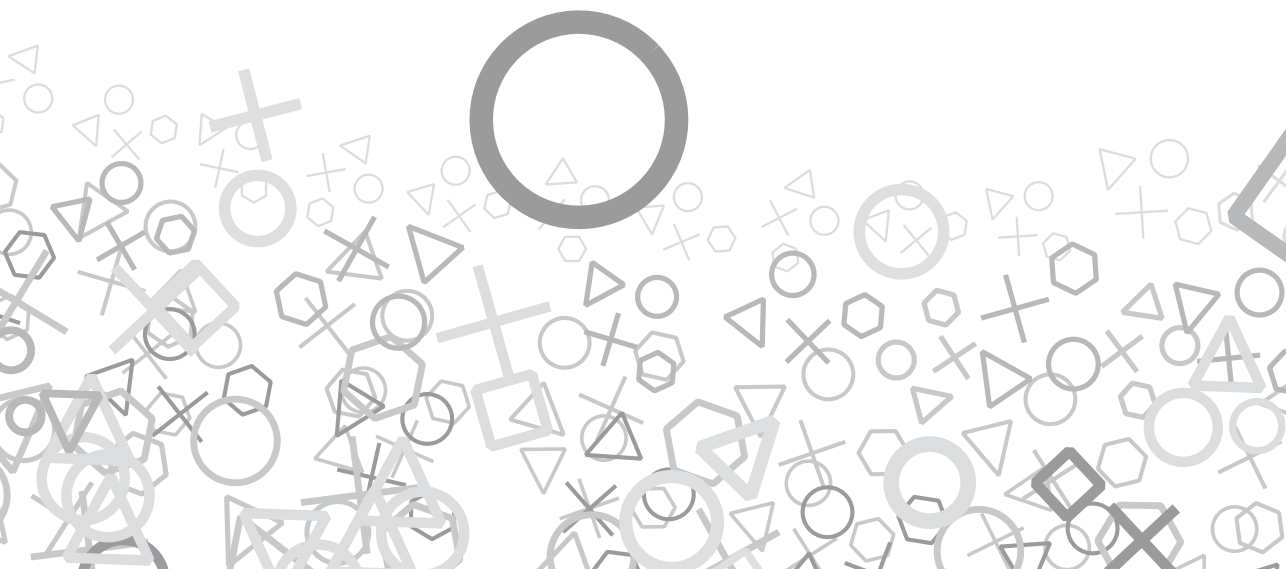



CHAPTER 6

The pharmacokinetics of the CYP3A substrate midazolam in morbidly obese patients before and one year after bariatric surgery

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ABSTRACT

Purpose

Bariatric surgery is nowadays commonly applied as treatment for morbid obesity (BMI >40 kg/m²). As information about the effects of this procedure on a drug's pharmacokinetics is limited, we aimed to evaluate the pharmacokinetics of CYP3A probe substrate midazolam after oral and intravenous administration in a cohort of morbidly obese patients that was studied before and 1 year post bariatric surgery.

Methods

Twenty morbidly obese patients (aged 26-58 years) undergoing bariatric surgery participated in the study of which 18 patients returned one year after surgery. 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. Midazolam concentrations were analyzed using population pharmacokinetic modeling.

Results

One year after bariatric surgery, systemic clearance of midazolam was higher [0.65 (7%) versus 0.39 (11%) L/min, mean ± RSE (P<0.01), respectively] and mean oral transit time (MTT) was faster [23 (20%) versus 51 (15%) minutes (P<0.01)], while oral bioavailability was unchanged (0.54 (9%)). Central and peripheral volumes of distribution were overall lower (P<0.05).

Conclusions

In this cohort study in morbidly obese patients, systemic clearance was 1.7 times higher one year after bariatric surgery, which may potentially result from an increase in hepatic CYP3A activity per unit of liver weight. Although MTT was found to be faster, oral bioavailability remained unchanged, which considering the increased systemic clearance implies an increase in the fraction escaping intestinal first pass metabolism.

INTRODUCTION

The prevalence of morbid obesity (body mass index, BMI >40 kg/m²) is increasing worldwide. In the United States, 6% of the population is morbidly obese while in Europe prevalence of obesity (BMI >30 kg/m²) ranges between 10 and 30% depending on the country¹⁻². Exact numbers on the prevalence of morbid obesity in Europe are lacking, but are estimated to range between 1-7%³⁻⁵.

Bariatric surgery is considered the most effective treatment for morbid obesity⁶⁻⁷. In 2011, more than 340,000 bariatric surgeries, including Roux and – Y gastric bypass (RYGB) and sleeve gastrectomy, were performed worldwide⁸. During a bariatric procedure, the stomach is reduced to a sleeve like structure or a small pouch and, in case of a Roux and –Y gastric bypass, the duodenum and initial part of the small intestine are bypassed⁹⁻¹¹. These alterations in the gastro-intestinal tract may cause an increase in stomach pH, an increase in gastric emptying time, and a decrease in the surface area of absorption^{9, 12-13}.

As such, a bariatric procedure may impact a drug's pharmacokinetics and have consequences for dosing. In particular for drugs undergoing CYP3A metabolism, it seems relevant to study the impact of bariatric surgery, as the CYP3A enzyme resides not only in the liver but also in the gut wall and is an important drug metabolising enzyme involved in the metabolism of approximately 25% of all clinically used drugs¹⁴. Besides bariatric surgery induced anatomical changes to the gastro-intestinal tract, the resulting reduction in (over)weight may also influence CYP3A activity itself¹⁵. It is well known that obese patients suffer from low-grade inflammation caused by macrophages and adipocytes in the adipose tissue which excrete inflammation markers and adipokines, including Il-6 and TNF-alpha¹⁶⁻¹⁸ which may lead to reduced CYP3A activity^{15, 19-20}. As studies in morbidly obese patients before and after bariatric surgery show a reduction in inflammation status in patients after bariatric surgery, it is hypothesized that CYP3A activity in patients after bariatric surgery recovers²¹⁻²². However, it seems that the inflammation status does not completely change back to non-obese (never been obese) individuals, as 6 months post surgery values of leptin, adiponectin and C-reactive protein (CRP) did not return to values found for lean (never been obese) patients²¹.

Midazolam is considered a model substrate drug for CYP3A activity as it is primarily metabolized by CYP3A²³. Therefore, in this study we aimed to evaluate the pharmacokinetics of midazolam after oral and intravenous administration in a cohort of morbidly obese patients that was studied before and 1 year post bariatric surgery. The results are used to evaluate consequences for dosing of midazolam in patients after bariatric surgery.

MATERIALS AND METHODS

Study design and patients

This is a prospective observational and interventional study in morbidly obese adult patients (NTC01519726, EudraCT 2011-003293-93). Before participation, all patients gave written informed consent. The study was approved by the local human research and ethics committee (VCMO, 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.

Morbidly obese patients undergoing a laparoscopic gastric bypass or sleeve surgery between 18 and 65 years were eligible for inclusion in the study. Patients were excluded if they used CYP3A inducing or inhibiting medication²⁴, used products containing grapefruit, wild grape, banpeiyu, pomegranate, star fruit or black berry within two weeks before the study, were pregnant, were breastfeeding, were younger than 18 or older than 60 years or suffered from renal insufficiency (eGFR MDRD4 <60 mL/min).

Study procedures

The study consisted of two occasions. The first occasion was on the day of laparoscopic bariatric surgery (occasion 1), of which the details and results have been described in a previous report²⁵. One year after bariatric surgery, the 20 patients who participated on occasion 1, were invited to participate in the second part of the study (occasion 2). The period of one year was chosen based on the Swedish Obese Subjects study showing a mean weight loss optimum of 32% of body weight 0.5-2 years after bariatric surgery²⁶.

For both occasions, patients fasted from midnight until the study started in the morning (typically at 09:00 o'clock) and were not allowed to eat or drink until 1 hour post intravenous midazolam dose. At first a 7.5 mg midazolam tablet was administered orally and after 160 ± 48 minutes an i.v. dose of 5 mg was administered. For the first occasion, the i.v. dose coincided with the induction of anesthesia for the bariatric surgical procedure while for the second occasion the i.v. dose was administered at 150 min after oral dose. Blood samples were collected at 5, 15, 30, 45, 55, 65, 75, 90, 120, 150 after oral dose and at 5, 15, 30, 60, 90, 120, 150, 180, 240 and 300, 390 and 510 minutes after intravenous dose at occasion 1, and at 5, 15, 30, 60, 90, 120, 150, 180, 240 and 300 minutes after intravenous dose at occasion 2. After collection, blood samples were centrifuged and plasma was stored at -80 °C until analysis. Samples were analyzed as described before²⁵. The Richmond Agitation Sedation Scale (RASS) was used to score the level of sedation in each participant after midazolam oral dose until administration of the intravenous dose²⁷.

Population pharmacokinetic analysis and internal validation

The population pharmacokinetic analyses was performed by means of nonlinear mixed effects modelling using NONMEM (version 7.2)²⁸; Pirana (2.7.1) and R (2.15) were used to visualize the data. Discrimination between different structural and statistical 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, 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 versus population-predicted concentrations plots) 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 model. The internal validity of the population pharmacokinetic model was assessed by the bootstrap re-sampling method using 500 replicates and normalized prediction distribution errors (NPDE)²⁹. Parameters obtained with the bootstrap replicates were compared with the estimates obtained from the original dataset and NPDE plots were checked for normal distribution characteristics and trends in the errors.

Midazolam concentration-time profiles were analysed separately (occasion 1, occasion 2) and simultaneously (occasion 1 and 2). The separate pharmacokinetic analyses allowed for initial exploration of the data and evaluation of covariate relationships within each population. For all analyses, two- and three compartment pharmacokinetics models were tested. For the description of the oral absorption phase, different models were tested including first order absorption, zero order absorption and a transit compartment model in which transit compartment rates (Ktr) were equalized to the absorption rate constant (Ka)³⁰. The mean oral transit time (MTT), which represents the average time for the drug from oral dose administration to appearance at the sample site, can be calculated from Ktr using $MTT = (N+1)/Ktr$ in which N is the number of transit compartments. For the statistical model, the individual parameter estimate (Empirical Bayes Estimate or post hoc value) of the *i*th individual was modelled according to (equation 1):

$$\theta_i = \theta_{mean} \times \exp(\eta_i) \quad (\text{Eq. 1})$$

where θ_{mean} is the population mean parameter value, 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. The residual variability, resulting from assay errors, model misspecifications and other unexplained sources, was best described with a proportional error model. The *j*th observed midazolam concentration of the *i*th patient (Y_{ij}) in equation 2:

$$Y_{ij} = C_{pred,ij} \times (1 + \varepsilon_{ij}) \quad (\text{Eq. 2})$$

where $C_{pred,ij}$ is the population 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 . Covariate analysis Covariates were plotted independently against the individual estimates of pharmacokinetic parameters to visualize potential relations. The following covariates were tested: body weight, BMI, lean body weight³¹, age, sex and bariatric surgery. The influence of the binary covariates bariatric surgery and sex was explored by means of estimating two separate thetas or by a factor ('Z') of increase/decrease according to equation 3:

$$P_i = P_p \times Z^{COVARIATE} \quad (\text{Eq. 3})$$

where P_i and P_p represent the individual and population parameter estimate, Z represents the factor for increase or decrease for the patients subgroup with a Z of 1 in case the covariate equals 0 or a Z of Z in case the covariate equals 1. In case the binary covariate bariatric surgery for a specific parameter improved the model significantly, it was evaluated whether this factor of increase or decrease could be related to the difference in body weight, lean body weight³¹ or BMI between occasion 1 and 2 using the following equations:

$$\text{If Occasion} = 1: P_i = P_p \quad (\text{Eq. 4})$$

$$\text{If Occasion} = 2: P_i = P_p \times \text{factor} \cdot (COV_{\text{BeforeSurgery}} - COV_{\text{AfterSurgery}}) \quad (\text{Eq. 5})$$

Furthermore, it was tested whether body weight, lean body weight, age or BMI was a linear (Eq. 7) or nonlinear (Eq. 8) covariate within occasion 1 or 2 using the following equations:

$$\text{If Occasion} = 1: P_i = P_p \quad (\text{Eq. 6})$$

$$\text{If Occasion} = 2: P_i = P_p \times (1 + W \times (COV - COV_{\text{median}})) \quad (\text{Eq. 7})$$

And/or:

$$\text{If Occasion} = 2: P_i = P_p \times \left(\frac{COV}{COV_{\text{median}}} \right)^x \quad (\text{Eq. 8})$$

where P_i and P_p represent individual and population parameter estimates, respectively; COV represents the covariate; COV_{median} represents the median covariate value; X represents the exponential scaling factor; and W represents the correlation factor between the population pharmacokinetic parameters and the covariate. The occasion conditions were switched vice versa to test covariate relationships within both groups.

Continuous covariates for both occasion 1 and 2 simultaneously were tested using linear and non-linear equations (equation 9 and 10).

$$P_i = P_p \times \left(\frac{COV}{COV_{median}} \right)^x \quad (\text{Eq. 9})$$

$$P_i = P_p \times (1 + W \times (COV - COV_{median})) \quad (\text{Eq. 10})$$

Potential covariates were separately entered into the model and statistically tested by use of the OFV and, if applicable, the 95% CI of the additional parameter. In addition, if applicable, it was evaluated whether the interindividual variability in the parameter concerned reduced in value upon inclusion of the covariate on the parameter. After forward inclusion ($p < 0.05$), a backward exclusion procedure was applied to justify the inclusion of a covariate ($p < 0.001$). The choice of the covariate model was further evaluated as discussed above (see Population pharmacokinetic analysis and internal validation section).

Model simulations

The final population pharmacokinetic model was used to simulate the midazolam concentration time curves after a 7.5 mg oral dose, a 5 mg intravenous dose and a 2.5 mg/h continuous infusion. Using Monte Carlo simulations, 1000 individuals were randomly generated based on body weight distribution of our study (144 ± 26 kg) and simulations based on theta and eta values of the final PK model were performed using NONMEM.

RESULTS

Patients and data

Of the 20 morbidly obese patients who participated in the first part of the trial (occasion 1), 18 patients returned 52 ± 3 weeks after bariatric surgery (occasion 2) and lost a mean of 44.5 ± 10.2 kg of body weight. Two of the 20 patients were lost to follow up to participate at occasion 2. Patients and study characteristics are summarized in Table 1. Figure 1 shows the midazolam concentration time values measured at both study occasions. At occasion 1, the occurrence of the peak concentrations after the i.v. dose were found to vary largely, which resulted from differences in time of administration of the intravenous midazolam. For post-bariatric surgery patients, the concentration time curves show a slightly earlier maximum concentration (C_{max}) after oral dose in comparison to morbidly obese patients before bariatric surgery, while in a few individuals of the morbidly obese patient group peak concentrations after the intravenous dose seemed higher.

Table 1 Patients and study characteristics (mean $\pm$ standard deviation)

	Morbidly obese patients before surgery (n=20)	Minimum-maximum	Patients after bariatric surgery (n=18 of 20)	Minimum-maximum
Female/Male	12/8		11/7	
Age (years)	43.6 $\pm$ 7.6	26 – 57	45.5 $\pm$ 7.4	27 – 58
Body weight (kg)	144.4 $\pm$ 21.7	112 – 186	98.3 $\pm$ 18.0	62 – 138
LBW (kg)	71.5 $\pm$ 11.9	53 – 95	59.5 $\pm$ 10.0	39 – 73
BMI (kg/m ²)	47.1 $\pm$ 6.5	40 – 68	31.9 $\pm$ 5.9	24 – 50
Weight loss (kg)	-	-	44.5 $\pm$ 10.2	21 – 58
Number of midazolam samples per patient	22 $\pm$ 3	13 – 24	21 $\pm$ 1	19 – 22
Gastric bypass/ sleeve gastrectomy	-	-	16/2	-
Time post surgery (weeks)	-	-	51.8 $\pm$ 2.5	49 – 57

BMI= body mass index; LBW= lean body weight

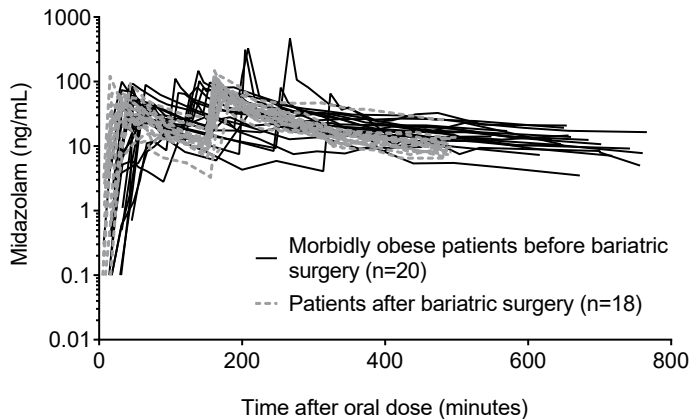


Figure 1 Midazolam concentration versus time after oral dose profiles upon a 7.5 mg oral midazolam dose and a 5 mg intravenous dose separated by 160 ± 48 minutes in 20 morbidly obese patients before (black lines) and 1 year after surgery (grey dotted lines). Two patients were unable to participate one year after surgery.

Pharmacokinetic analysis

For the population pharmacokinetic analysis including the data of both occasions a three compartment model, in which the second peripheral volume was a fraction of the first peripheral compartment best described the data. Midazolam oral absorption was best described using 5 transit compartments, while the addition of more transit compartments did not further improve the fit of midazolam concentrations after both oral and intravenous administration. Table 2 shows the parameters estimates of the simple pharmacokinetic model without covariates.

Table 2 Parameter values of the simple (without covariates) and final (with covariates) population pharmacokinetic models and results of the bootstrap analysis.

Parameter	Simple model of simultaneous analysis	Final model of simultaneous analysis	Bootstrap of final simultaneous model
	Value (RSE)	Value (RSE)	Median (2.5 - 97.5 percentile)
<i>Fixed effects</i>			
Ka = Ktr	0.199 (11%)	-	-
Ka = Ktr _{Morbidly obese} (min ⁻¹)	-	0.117 (15%)	0.114 (0.08-0.15)
Ka = Ktr _{Bariatric patients} (min ⁻¹)	-	0.267 (19%)	0.263 (0.08-0.45)
F	0.560 (10%)	0.537 (9%)	0.543 (0.44 - 0.63)
CL (L/min)	0.381 (26%)	-	-
CL _{Morbidly obese} (L/min)	-	0.385 (11%)	0.366 (0.29-0.48)
fCL _{Bariatric patients} (L/min)	-	1.68 (7%) (* CL _{morbidly obese} = 0.647)	1.70 (1.18-2.18) (* CL _{morbidly obese} = 0.634)
Q (L/min)	0.888 (21%)	-	-
Q _{Morbidly obese} (L/min)	-	0.669 (24%)	0.764 (0.11-1.23)
fQ _{Bariatric patients} (L/min)	-	3.22 (32%) (* Q _{Morbidly obese} = 2.15)	2.907 (-22.6-29.0) (* Q _{Morbidly obese} = 3.71)
Q2	0.644 (21%)	0.551 (23%)	0.548 (0.25-0.86)
V _{central} (L)	54.7 (17%)	-	-
V _{central Morbidly obese} = V _{median BW} * (1+X*(BW-median BW))			
V _{median BW}	-	37.3 (18%)	37.2 (17.8-56.8)
X	-	0.0435 (92%)	0.052 (-0.42-0.51)
V _{central Bariatric patients} (L)	-	37.3 (18%)	37.2 ()
V _{1st peripheral} (L)	247 (30%)	-	-
V _{Peripheral Morbidly obese} = V _{median BW} * (BW/median BW) ^Y			
V _{median BW}	-	106 (17%)	113 (20.9-190.3)
Y	-	3.93 (20%)	3.99 (1.9-5.9)
V _{1st peripheral Bariatric patients} (L)	-	106 (17%)	113 (1.9-5.9)
fV _{2nd peripheral}	0.169 (25%) (*V _{1st peripheral} = 42 L)	0.359 (27%) (*V _{1st peripheral} = 38 L)	0.311 (0.13-0.58) (*V _{1st peripheral} = 40 L)
<i>Interindividual variability (%)</i>			
Ktr=Ka	50 (17%)	42.4 (15%)	40.6 (27-54)
CL	41.5 (24%)	19.7 (38%)	17.7 (-14-32)
F	28.6 (23%)	33.4 (18%)	32.6 (16-45)
V _{central}	60.8 (20%)	53.7 (39%)	54.3 (-49-102)
V _{1st peripheral}	0 FIX	0 FIX	0 FIX
<i>Proportional residual error (%)</i>			
	46.2 (6%)	42.1 (5%)	41.0 (12.2)
OFV	6218	5885	5997 (804)

BW= body weight (median=127 kg for all data), CL=Clearance (L/min), F= Oral bioavailability, fCL_{Bariatric patients} (L/min)= fraction of midazolam clearance of morbidly obese patients to estimate bariatric patient clearance, fQ_{Bariatric patients} = fraction of intercompartmental clearance of morbidly obese patients to estimate intercompartmental clearance of bariatric patients, fV_{peripheral}= fraction of first peripheral volume of distribution to estimate second peripheral volume, Ktr= transit compartment rate (min⁻¹), Ka= oral absorption rate (min⁻¹), OFV= Objective function value (-2LL), Q=intercompartmental clearance (L/min), RSE(%)=relative standard error, V=Volume of distribution (L), V_{Peripheral}= first peripheral volume of distribution.

In the covariate analysis, the binary covariate ‘bariatric surgery’ proved an important covariate for clearance (CL), oral absorption rate (Ka), inter compartmental clearance (Q) and volumes of distribution (V). For clearance, the covariate bariatric surgery gave the largest drop in OFV (-91 Δ OFV, $p < 0.001$), while a linear covariate relation with body weight resulted in a drop in OFV (-80 Δ OFV). After bariatric surgery, clearance was 1.68 times higher than in morbidly obese patients before surgery, while the extent of this increase could not be related to the loss in (lean) body weight ($p > 0.05$, table 2). Bariatric surgery as covariate on Ka also resulted in an improved fit of the model (-167 Δ OFV,

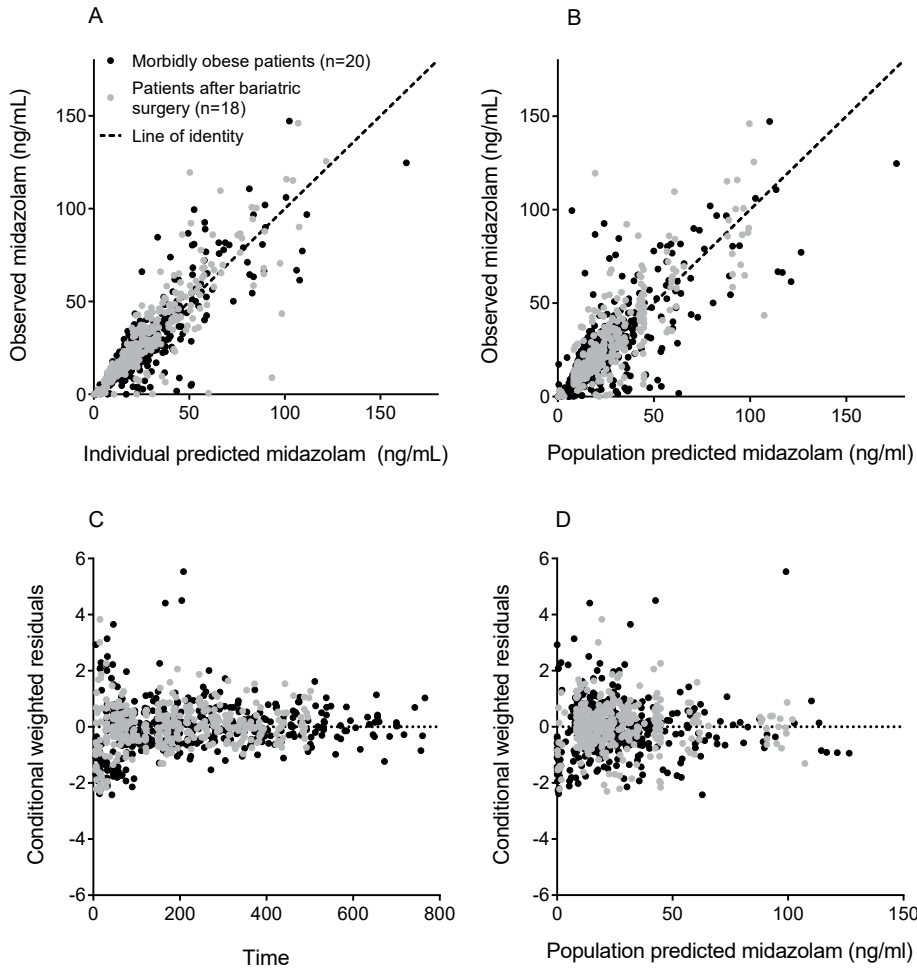


Figure 2 Observed versus individual predicted midazolam concentrations (A), observed versus population predicted midazolam concentrations (B), conditional weighted residuals (CWRES) versus time in minutes (C) and population predicted midazolam concentrations (D) of the final model for 20 morbidly obese patients (black dots, occasion 1) of which 18 returned 1 year post surgery for a second study visit (grey dots, occasion 2). The dashed line represents the line of identity ($x=y$).

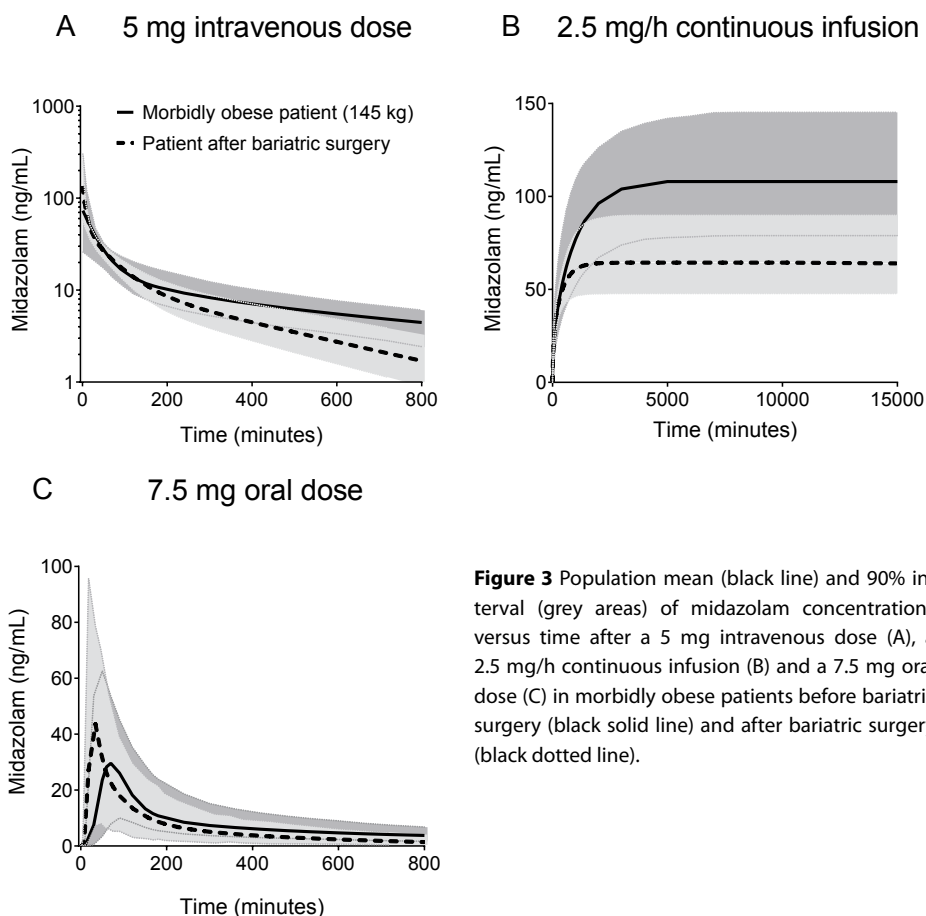


Figure 3 Population mean (black line) and 90% interval (grey areas) of midazolam concentrations versus time after a 5 mg intravenous dose (A), a 2.5 mg/h continuous infusion (B) and a 7.5 mg oral dose (C) in morbidly obese patients before bariatric surgery (black solid line) and after bariatric surgery (black dotted line).

$p < 0.001$). In the final model, K_a was separately estimated for both occasions and was found to have a larger value in patients after bariatric surgery (0.117 versus 0.267 min^{-1} , Table 2). As a consequence, the mean oral transit time (MTT), which is calculated from the oral absorption rate, was 51.3 (15%) before versus 22.6 (19%) minutes after bariatric surgery. Furthermore, bariatric surgery resulted in a 3.22 times increase in inter compartmental clearance, Q (0.669 to 2.15 L/min , $-16 \Delta\text{OFV}$, $p < 0.001$). Finally, the central and the peripheral volumes of distribution were overall lower in patients after bariatric surgery without a significant influence of (lean) bodyweight within this group. In the morbidly obese patients group, central and peripheral volume increased with body weight ($-6 \Delta\text{OFV}$, $p < 0.02$ and $-68 \Delta\text{OFV}$, $p < 0.001$, respectively, Table 2). As the second peripheral volume of distribution was modeled as a fraction of the first peripheral volume of distribution, for morbidly obese patients before surgery it varied with body weight in a similar manner as the first peripheral volume of distribution (Table 2). Concerning oral bioavailability (F) and inter-compartmental clearance to the second peripheral com-

partment (Q2) none of the covariates were of significant influence ($p>0.05$). Parameters estimates of the final pharmacokinetic model are shown in Table 2 and goodness of fit plots are shown in Figure 2. A 500 replicate bootstrap showed validity of the model (94% successful, Table 2) and NPDE plots are presented in the electronic supplementary material (ESM 1, see end of this Chapter) and showed a normal distribution of errors without any trends for both occasions.

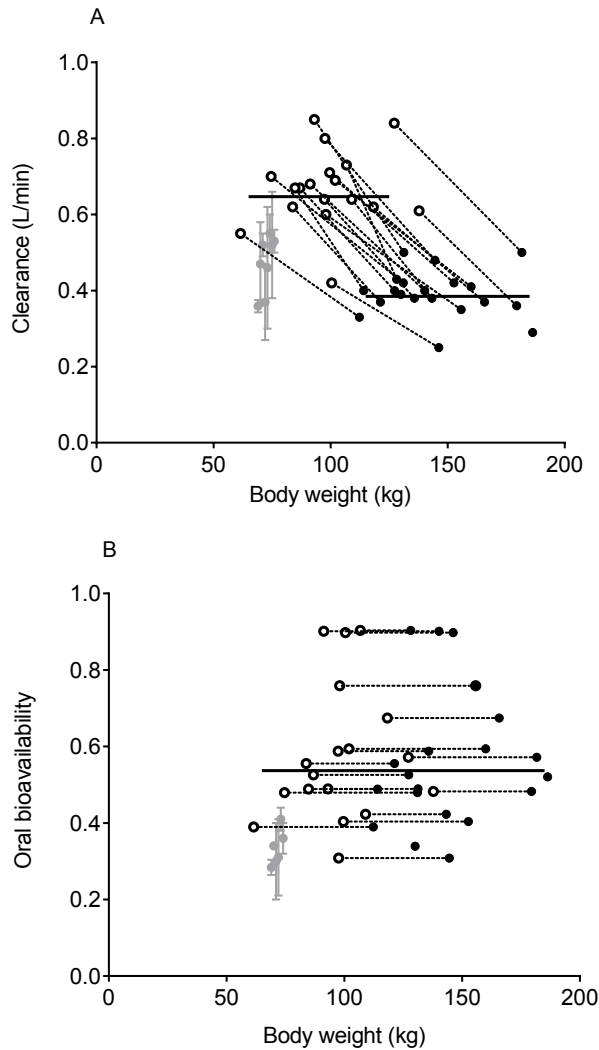


Figure 4 Empirical bayes estimates (black dots) and population mean estimates (black lines) of midazolam clearance (A) and oral bioavailability (B) of the final pharmacokinetic model in 20 morbidly obese patients (black closed dots) and 18 bariatric patients (black open dots) versus body weight (kg). Parameter values found for healthy volunteers studies from the literature were added for comparison (grey dots)^{25, 34-40}.

In Figure 3 the population mean and 90% confidence interval of 1000 Monte Carlo midazolam dose simulations for morbidly obese patients before and after surgery are presented. After a 5 mg intravenous dose, midazolam concentrations in a bariatric surgery patient show a higher initial midazolam concentration and a faster decline over time compared to a morbidly obese patient before surgery (Figure 3A). Upon a midazolam 2.5 mg/h continuous infusion a bariatric patient is exposed to a lower steady state concentration in comparison to a morbidly obese patient (Figure 3B), while steady state concentrations are reached approximately 2.5 times faster in bariatric patients (~14 h) than in morbidly obese patients (~37 h). Finally, oral midazolam in a bariatric patient will result in a shorter time to maximum concentration (T_{max} , 32 versus 65 minutes) and 1.5 times increase in midazolam maximum concentration (C_{max}) in comparison to before surgery (Figure 3C).

DISCUSSION

In this cohort study in which morbidly obese patients are studied until one year after bariatric surgery, we aimed to determine how and to what extent midazolam pharmacokinetics after oral and intravenous administration are affected by bariatric surgery. One year post bariatric surgery, we found that midazolam systemic clearance and mean oral transit time were substantially increased while oral bioavailability remained unchanged. Central and peripheral volumes of distribution were generally lower in patients after bariatric surgery. The main finding of this study is the substantial increase in midazolam systemic clearance in all 18 patients one year after bariatric surgery compared to their values before surgery. This increase in clearance after bariatric surgery could not be contributed to the decrease in body weight as the body weight model was inferior to the bariatric surgery model ($p < 0.05$), (Figure 4). Hepatic CYP3A protein expression in liver biopsies has been reported to be unaltered after bariatric surgery indicating unchanged CYP3A mediated clearance³². However, Tandra *et al.* also found increased systemic clearance of midazolam in 18 bariatric patients >1 year post RYGB surgery in comparison with 18 controls (1.57 ± 0.95 versus 0.92 ± 0.72 L/min, $p = 0.03$)³³. In their study, control patients were matched for age, sex, race, and body mass index, while in our study we compared midazolam pharmacokinetics within the same cohort using a follow up design. Comparing our values to systemic clearance values in healthy volunteers (Figure 4A), it seems that systemic clearance values post bariatric surgery are higher than those of healthy volunteers found in the literature^{25, 34-40}. We anticipate that the increase in systemic midazolam clearance may be explained by a recovery of hepatic CYP3A activity due to decreased inflammation status, as many studies have shown a reduction in inflammatory adipokines in the plasma of patients after bariatric surgery²².

Moreover, it has been shown in *in vitro* and animal studies that a fatty liver, which is highly associated with morbid obesity, represses CYP3A activity⁴¹⁻⁴². While CYP3A activity may have recovered one year after bariatric surgery, Immonen *et al.* showed on the other hand that 6 months after bariatric surgery both the fat content and size of the liver is reduced to almost the level of lean subjects, which could imply a reduced clearance²¹. The fact that we identify in our study an increase in systemic midazolam clearance post bariatric surgery implies that the increased CYP3A activity per unit of liver compensates and surpasses the reduction in liver size that is associated with bariatric surgery in these patients. Another explanation could be a recovery in total liver blood flow, due to recovery of fatty liver and/or steatosis⁴³, but as midazolam is an intermediate extraction ratio drug this seems unlikely⁴⁴.

Midazolam mean oral transit time was twice as fast in patients one year post bariatric surgery in comparison to before surgery. Decreased T_{max} in bariatric surgery patients has been reported before for oral caffeine, tolbutamide, midazolam, omeprazole and duloxetine administration^{33,45}. The faster midazolam oral absorption may be explained by faster gastric emptying of the stomach due to the reduced stomach size^{12-13, 46-47}. In contrast to more rapid oral absorption, for oral bioavailability, we found no difference before and after bariatric surgery. From a comparison to healthy volunteers (Figure 4B), it can be concluded that oral bioavailability values in bariatric patients do not seem to return to values found for healthy volunteers, but remains at the level of those found for morbidly obese patients. The oral bioavailability value (F) 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 can be assumed to be equal to 1 in morbidly obese patients before and after surgery⁴⁸. In addition, assuming no change in hepatic blood flow and blood to plasma partition ratio before and after surgery, F_H will decrease approximately 1.68 times post bariatric surgery as a result of 1.68 times increased systemic clearance. So, given the unchanged total bioavailability, F_{total} , we identified in our study, this implies that the midazolam fraction escaping gut wall (F_g) increases 1.68 times one year after bariatric surgery. An increase in F_g was also predicted by Darwich *et al.*, who showed that post RYGB surgery the F_g of CYP3A substrate simvastatin increased with 13%⁴⁹. Increased F_g may be due to the bypass of the intestines resulting from this type of surgery, in which normally approximately 75% of the midazolam dose would have been absorbed^{48,50}. Another explanation could be an increase in splanchnic blood flow resulting in an increase in F_g , however this seems very unlikely in view of the decrease in bodyweight associated with bariatric surgery and therefore an anticipated decrease in splanchnic blood flow instead of increase.

For midazolam central and peripheral volume of distribution we observed overall lower values in post bariatric surgery patients without variation due to body weight

($p > 0.05$, Table 2). While we anticipate that this is due to the smaller range in bodyweight in the bariatric patient group, as within the morbidly obese patient group volume of distribution was highly depended on body weight as was reported before²⁵. To account for the influence of body weight on both the first and second peripheral volume of distribution, the second peripheral volume was modeled as a fraction of the first volume of distribution. The general reduction in volume of distribution after bariatric surgery may result from weight loss resulting in substantial reductions in blood volume and adipose tissue⁵¹.

Although this study provides unique information on the pharmacokinetics of midazolam after both oral and intravenous dose administration in a new and emerging patient population, the study may have some limitations. First, 2 of the 18 patients underwent a sleeve gastrectomy procedure, which is an insufficient number to draw any conclusion on the effect of a sleeve gastrectomy on midazolam pharmacokinetics. However, these 2 patients did show a major loss in body weight to an extent that was similar to that of the 16 gastric bypass patients, which was the reason why we included these patients in the analysis. Moreover, when excluding these two patients from the dataset, none of parameter estimates were significantly different (data not shown). Second, at occasion 1, patients underwent surgery and anesthesia, which was not the case during occasion 2. This may potentially have influenced the results on midazolam PK we report in this study. It is well known that during a surgery cardiac output is lowered which may have caused lower midazolam clearances for morbidly obese patients. However, the duration of surgery and anesthesia was quite limited (86.4 ± 31 minutes) in comparison to the study period of the first occasion (~ 660 minutes), minimizing the influence of surgery. Moreover, bariatric surgery was performed using minimally invasive techniques (laparoscopic techniques) reducing hemodynamically induced changes. Furthermore, surgery was performed 159 ± 67 minutes after oral midazolam dose administration, which excludes any influence of surgery/anesthesia on midazolam the oral absorption phase. For these reasons, we think that the short duration of surgery/anesthesia during the first occasion is not of significant influence on the conclusion drawn based on these data.

The midazolam dose simulations provide insight in how the altered pharmacokinetics in bariatric patients affect midazolam concentration time profiles after oral or intravenous administration. A 5 mg intravenous midazolam bolus dose results in higher initial midazolam concentrations in a patient post bariatric surgery than in a morbidly obese patient. This indicates that, in comparison to morbidly obese patients, a lower intravenous bolus dose may be anticipated in patients post bariatric surgery, as after a intravenous bolus dose, midazolam effect is primarily determined by the initial concentrations. For an continuous intravenous infusion, a lower steady state concentration is reached in a bariatric patient due to the almost doubled midazolam clearance value compared to morbidly obese patients. So to reach a similar steady state concentration in a bariatric

patient a higher mg/h dose seems necessary. In this respect, it is important to realise that a post-bariatric surgery patient does not only need a higher dose than before surgery but also may need a higher dose in mg/h than a non-obese patient given the differences in clearance values (Figure 4). Furthermore, the steady state concentration is reached 2.5 times faster in a bariatric surgery patient compared to a morbidly obese patient. Finally, a midazolam oral tablet will result in increased $C_{\max}$ and earlier $T_{\max}$ in a bariatric patient.

Finally, the influence of bariatric surgery on midazolam systemic clearance found in this study may be extrapolated to other drugs which are also primarily metabolised by CYP3A, as midazolam is considered a CYP3A probe substrate²³. While the extrapolation potential depends on many factors, including extraction ratio and physico-chemical properties of the drug, it may be speculated that other major CYP3A substrates may show a similar effect of bariatric surgery on systemic clearance.

In conclusion, in this cohort study in morbidly obese patients, systemic clearance was 1.7 times higher one year after bariatric surgery, which may potentially result from an increase in hepatic CYP3A activity per unit of liver. Even though mean oral transit time was found to be faster, oral bioavailability remained unchanged, which considering the increased systemic clearance implies an increase in the midazolam fraction escaping intestinal first pass metabolism after an oral administration. In patients after a bariatric surgery, these alterations will result in lower midazolam steady state concentrations and in higher and earlier peak concentrations after oral administration in comparison to morbidly obese patients before bariatric surgery.

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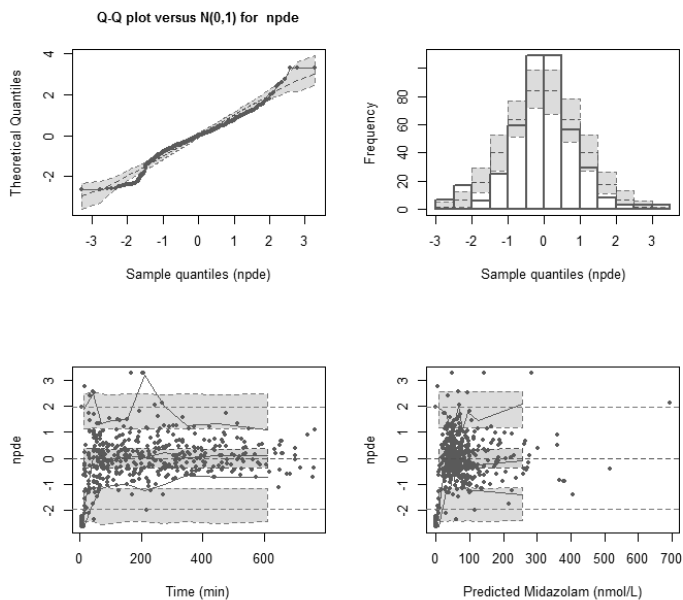
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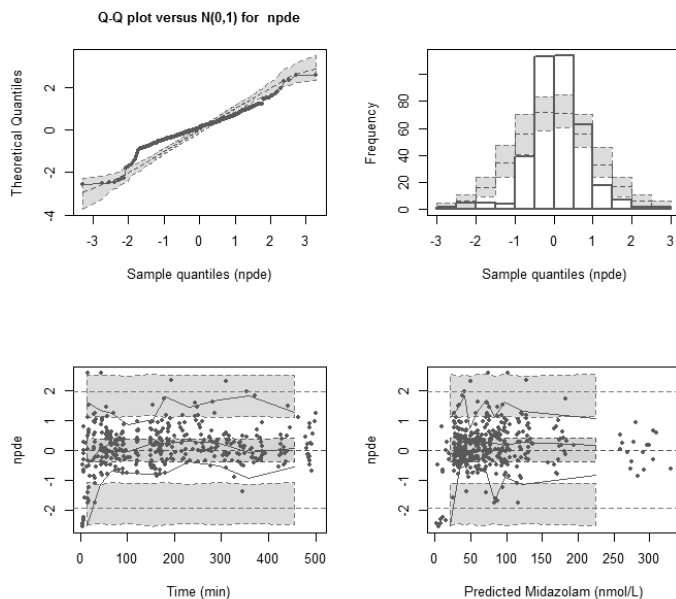
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SUPPLEMENTARY MATERIAL TO CHAPTER 6



ESM 1 A Normalised prediction distribution errors plots of the final pharmacokinetic model of midazolam concentrations in 20 morbidly obese patients before bariatric surgery.



ESM 1 B Normalised prediction distribution errors plots of the final pharmacokinetic model of midazolam concentrations in 18 patients whom returned one year after bariatric surgery.

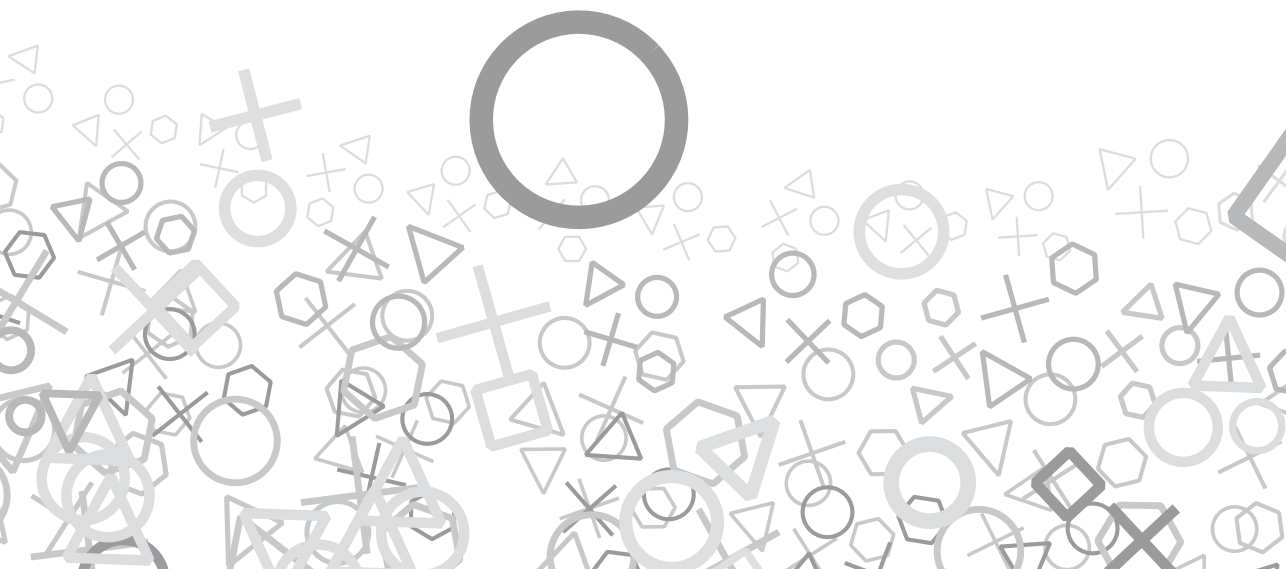


CHAPTER 7

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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Under review at CPT:PSP



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

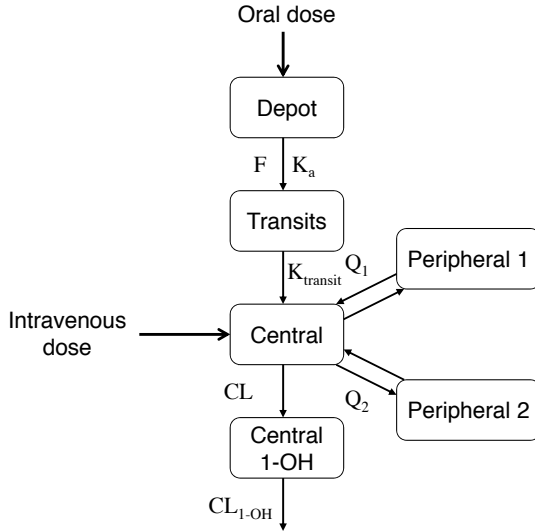
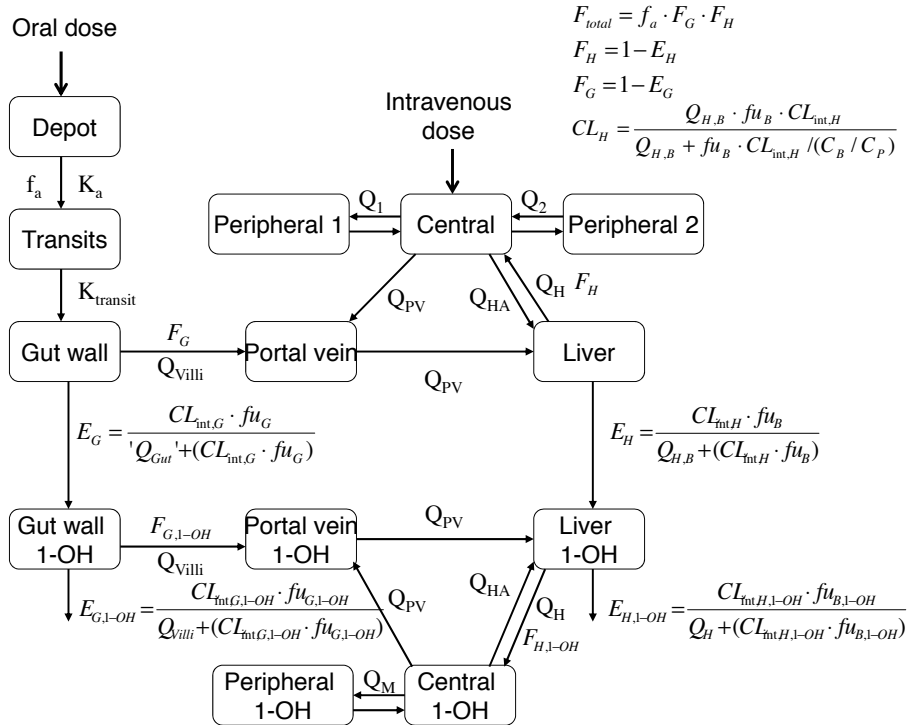


Figure 1 Schematic representation of the intermediate population pharmacokinetic model (a) and semi-PBPK model (b) for midazolam and its 1-OH-midazolam metabolite (1-OH). B= blood; CL_{int} = intrinsic clearance; E= extraction ratio; G= gut wall; F= bioavailability; f_s = fraction absorbed into the gut 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:

$$\theta_i = \theta_{mean} \times e^{\eta_i} \tag{Eq. 8}$$

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.

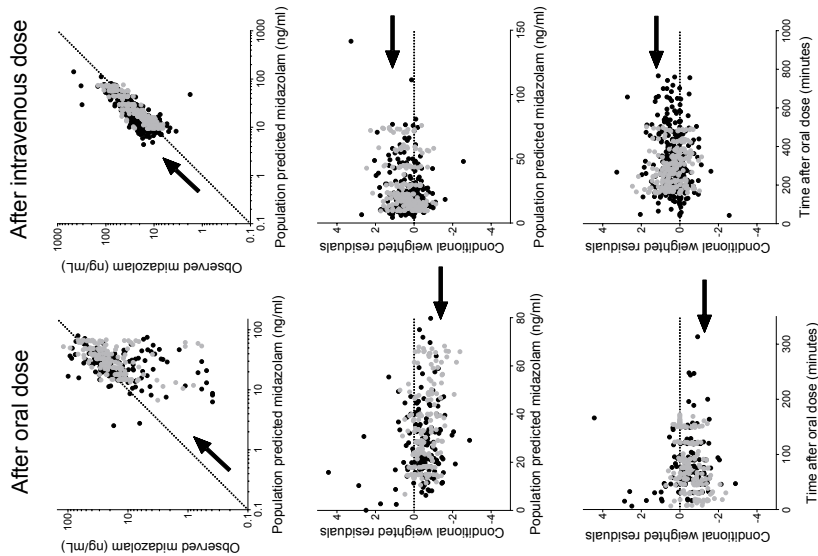
Potential covariates were separately entered into the model and statistically tested ($p < 0.05$) by use of the OFV and, if applicable, the 95% confidence interval of the additional parameter. In addition, if applicable, it was evaluated whether the inter-individual variability (eta) in the parameter 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

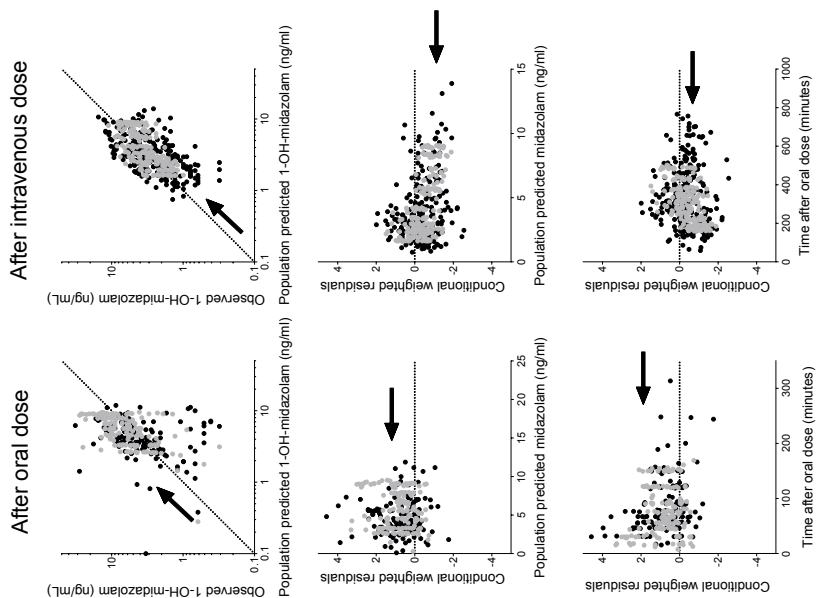
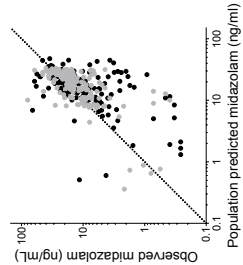


Figure 2 (a) Goodness-of-fit plots for midazolam (left) and 1-OH-midazolam (right) plasma concentrations for the population PK model (intermediate model, Figure 1a) for morbidly obese (black dots) and weight loss patients (grey dots), including population predicted versus observed plots (upper row), 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

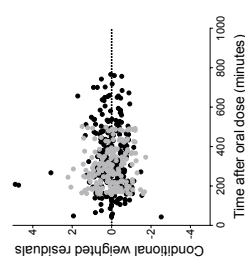
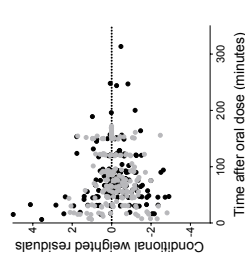
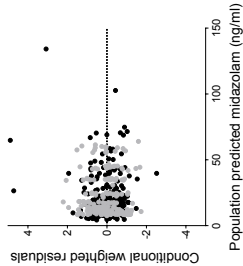
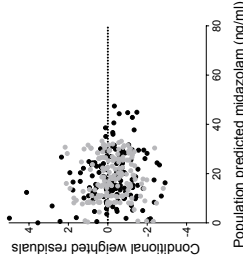
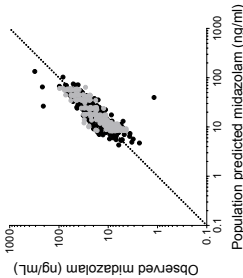
b

Midazolam

After oral dose

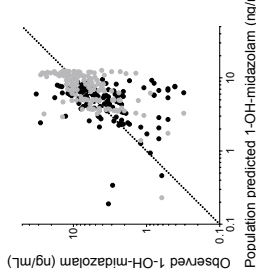


After intravenous dose

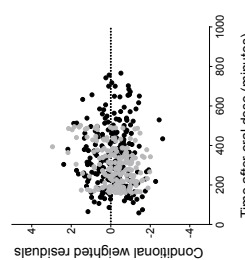
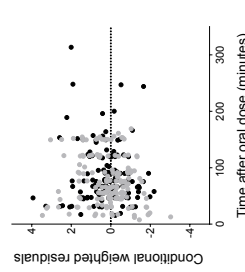
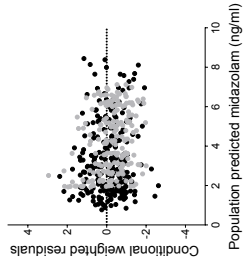
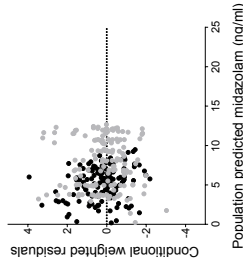
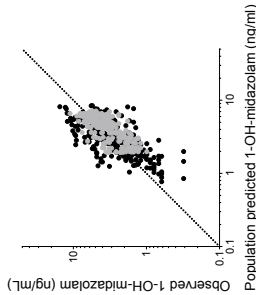


1-OH-midazolam

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

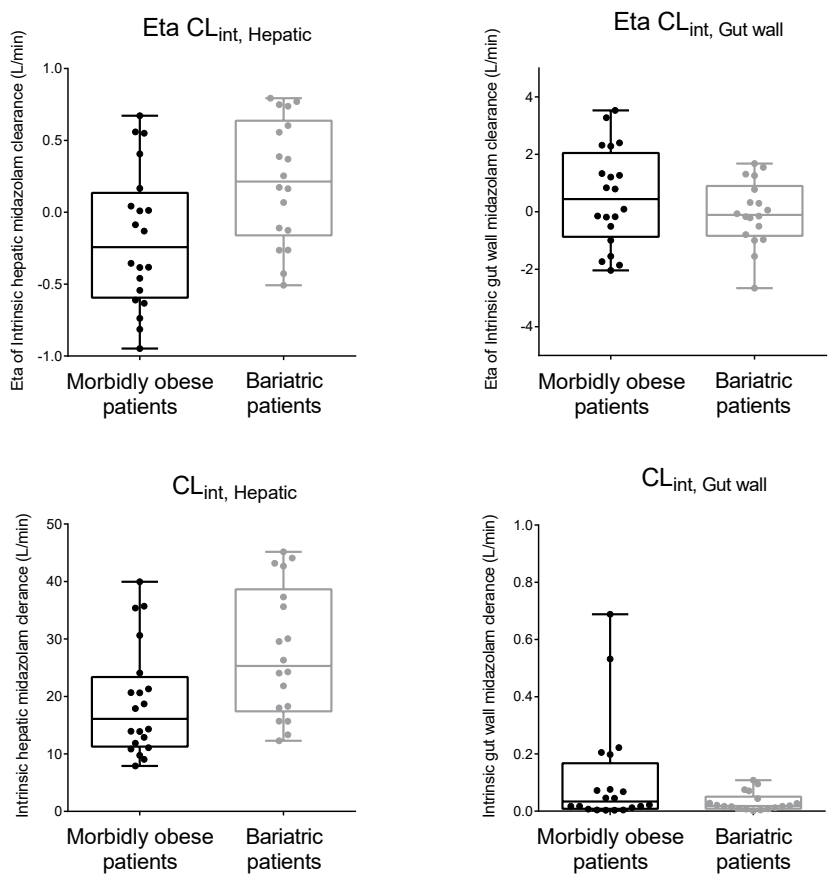


Figure 3 Box and whisker plots of eta and post hoc parameter estimates before addition of covariate effects for intrinsic hepatic (CL_{int,Hr}, left panels, shrinkage of 1%) and gut wall (CL_{int,Gr}, right panels, shrinkage of 21%) midazolam clearance in morbidly obese patients before (black) and after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

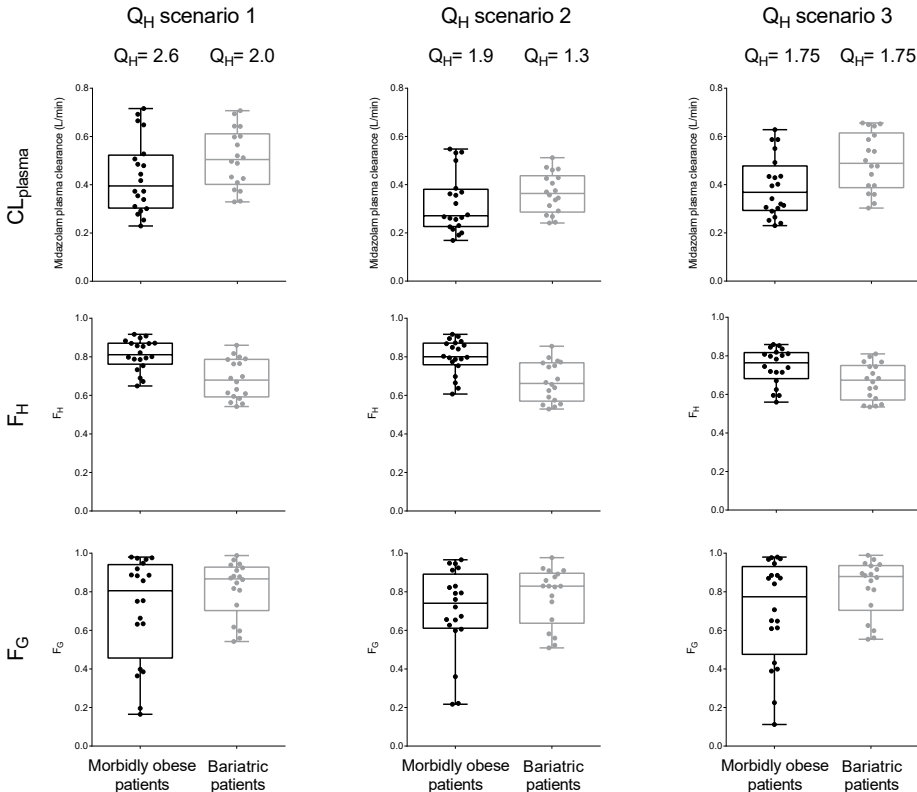


Figure 4 Box and whisker plots of calculated midazolam plasma clearance (equation 7, upper panels), F_H (equation 1 and 2, middle panels) and F_G (equation 3 and 5, lower panels) for morbidly obese (black) and weight loss patients (grey) for three different blood flow scenarios (Q_H in L/min, Table 1). 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-

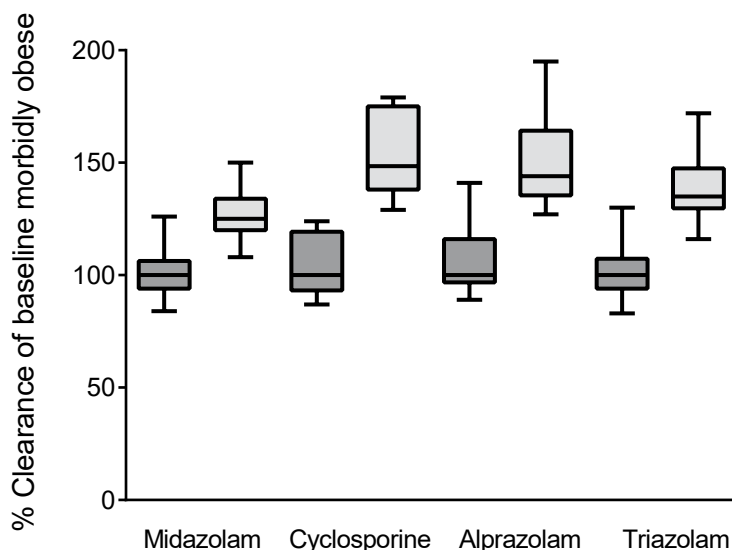


Figure 5 Box and whisker plots of simulated baseline systemic plasma clearances for the SimCYP morbidly obese patient population (dark grey boxes) and percentage change from baseline when hepatic CYP3a abundance is increased by 1.5 times for the morbidly obese population in the SimCYP simulator (light 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 well²⁶, 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/min²⁵. 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 substrates³⁷. 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 authors ² 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

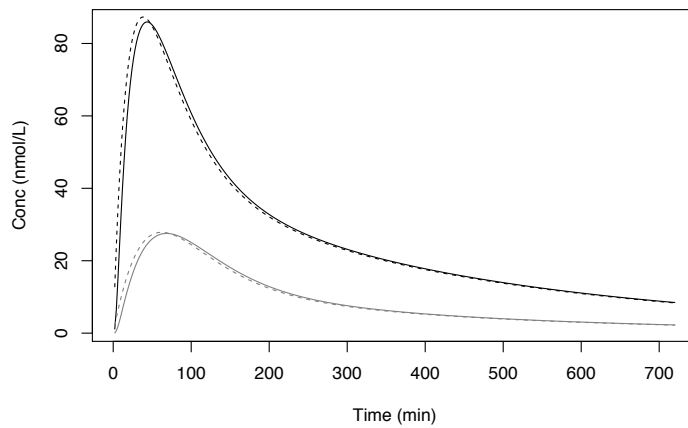


Figure 1 A comparison of simulated concentrations from the original model (solid lines) and the reduced model (dashed lines). Black lines indicate midazolam concentrations and grey lines indicate 1-OH-midazolam concentrations.

REFERENCES

1. Yang, J. et al. Prediction of intestinal first-pass drug metabolism. *Curr Drug Metab.* 2007; 8 (7), 676-684.
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

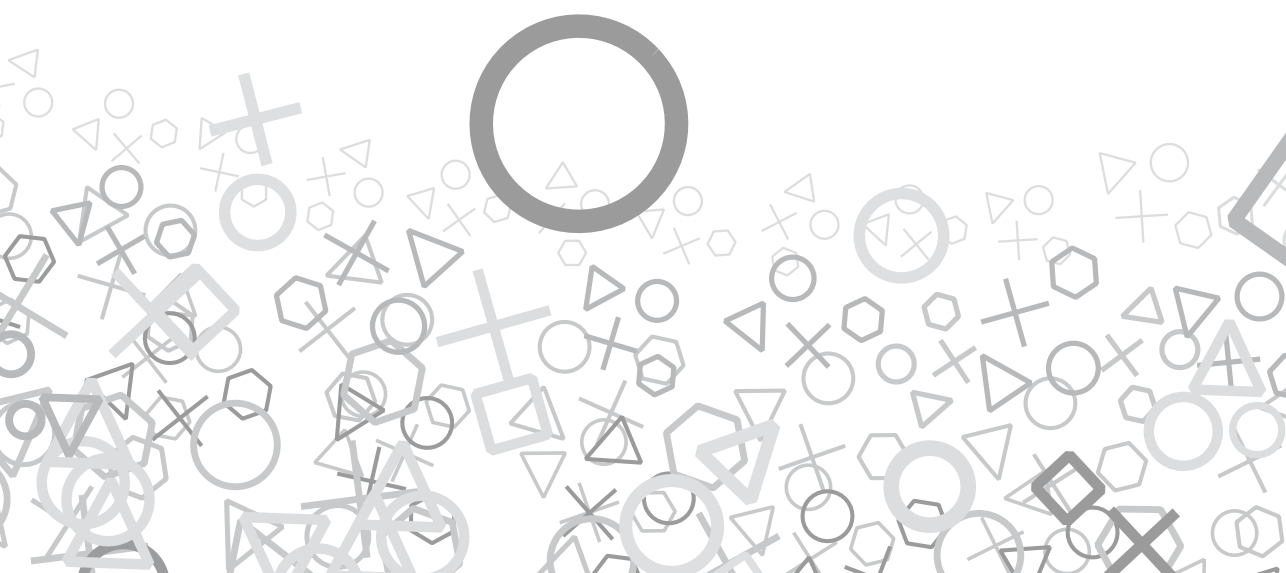
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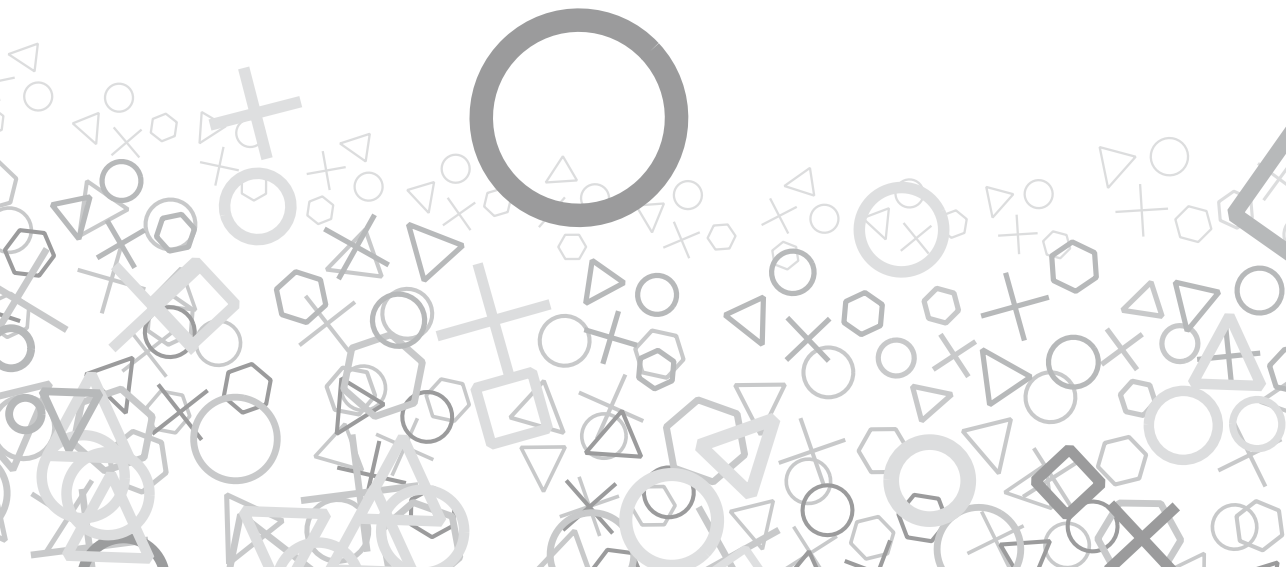
Summary, Conclusions and Perspectives





CHAPTER 8

Concepts and applications for evidence-based dosing in morbidly obese patients before and after weight loss surgery: Summary, conclusion and perspectives



SUMMARY AND CONCLUSIONS

Introduction and background

The prevalence of morbid obesity (body mass index, BMI > 40 kg/m²) is increasing across the globe¹⁻². The physiological changes associated with morbidly obese patients may impact the pharmacokinetics (PK) and pharmacodynamics (PD) of drugs and thus drug exposure and effects. Therefore (clinical) studies guiding evidence-based dosing in the morbidly obese population are needed, particularly in view of the increased risk of (morbidly) obese patients to develop serious comorbidities including cancer, diabetes type 2, cardiovascular diseases, etc.³. Currently, knowledge to what extent these physiologic changes influence absorption, metabolism, distribution, elimination and ultimately efficacy and safety of drugs is largely unknown. While until today studies on drug pharmacokinetics in obesity predominantly included overweight (BMI 25-30 kg/m²) and moderately obese patients (BMI 30-40 kg/m²), there is a strong need for pharmacokinetic studies in morbidly obese patients. In the end, the influence of (morbid) obesity on the PK-PD relationship should be characterized to guide dosing in this population.

Furthermore, as a result of an increase in the number of morbidly obese patients, also the number of patients who undergo weight loss or bariatric surgery is increasing⁴. Bariatric surgery or weight loss surgery is considered the most effective treatment option for morbid obesity⁵⁻⁶ and results, among other factors, in long term weight loss, remission of type 2 diabetes and overall mortality⁶⁻⁷. Bariatric patients present physicians and pharmacists with many challenges regarding safe and effective drug therapy, as bariatric procedures may impact a drug's pharmacokinetics both due to the anatomical changes made to the gastro-intestinal tract and the induced loss in body weight. On average, bariatric patients lose a mean of 32% of total body weight 0.5-2 years after the bariatric procedure⁶. For these reasons, also for the bariatric patient population, insight into changes in PK and PD that can be expected and evidence-based dosing recommendations are needed.

As a first step, **Chapter 2** provides an overview of findings reported in pharmacokinetic studies in both obese and non-obese subjects which are sorted by the metabolic or elimination pathway of the drug⁸. This overview shows that the impact of obesity on drug metabolism and elimination seems to depend on the metabolic or elimination pathway primarily involved in the clearance of a drug. It was shown that Cytochrome P450 3A (CYP3A) metabolized drugs have lower total (oral) clearance values, while clearance of drugs primarily metabolized by uridine diphosphate glucuronosyltransferase (UGT), glomerular filtration and/or tubular-mediated mechanisms, xanthine oxidase, N-acetyltransferase or CYP2E1 appears higher in obese versus non-obese patients.

Furthermore, in **Chapter 3** an overview of the impact of obesity on each aspect of a drug's pharmacokinetics as well as perspectives for future research into the influence of obesity on pharmacokinetics are summarized⁹. This overview shows that (morbid) obesity may substantially impact the distribution of drugs, while the magnitude and direction of change are difficult to predict based on the lipophilicity of the drug alone. Relative to the influence of obesity on distribution, the impact of (morbid) obesity on clearance may be smaller and more predictable based on the elimination pathway involved (Chapter 2). Finally, Chapter 3 shows that very little is known about the influence of (morbid) obesity on oral absorption and bioavailability, while from a small number of studies it seems that oral drug absorption may be altered.

Given the lack of information on drug absorption, distribution and clearance in morbidly obese and bariatric surgery patients, we decided to study two different drugs in these populations in this thesis. First, we studied cefazolin subcutaneous tissue penetration in morbidly obese patients undergoing bariatric surgery. In addition, we evaluated the impact of both morbid obesity and bariatric surgery on the pharmacokinetics of CYP3A substrate midazolam after semi-simultaneous oral and intravenous administration.

Influence of morbid obesity on cefazolin pharmacokinetics

Cefazolin is a first generation cephalosporin antibiotic which is widely applied for the prevention of surgical site infections during many types of surgical interventions, including bariatric surgery¹⁰. Cefazolin is eliminated by glomerular filtration and active tubular excretion¹¹. Studies report more surgical wound infections in morbidly obese patients, while cefazolin plasma concentrations seem to reach adequate levels in morbidly obese patients¹²⁻¹³. Yet, for morbidly obese patients it was unknown whether adequate levels of cefazolin were reached at the target site, which in this case is the interstitial space fluid (ISF) of the subcutaneous adipose tissue around the surgical wounds (abdomen). Therefore, in **Chapter 4** we aimed to measure and compare unbound cefazolin concentrations in the ISF of the subcutaneous adipose tissue of morbidly obese and non-obese patients. The results were used to quantify the influence of morbid obesity on cefazolin pharmacokinetics in the subcutaneous adipose tissue taking into account protein binding of this drug¹⁴.

After a 2 gram cefazolin intravenous bolus dose, total and unbound cefazolin plasma concentrations were collected in nine morbidly obese (141 ± 22 kg, 107-175 kg) and 7 non-obese patients (86 ± 13 kg, 72-109 kg). In addition, using clinical microdialysis, unbound cefazolin ISF concentrations of the abdominal adipose tissue were collected until 4 hours after dosing. It was found that unbound cefazolin subcutaneous tissue penetration, defined by the unbound AUC ratio ($fAUC_{\text{tissue}}/fAUC_{\text{plasma}}$) was lower in morbidly obese compared with non-obese patients (0.70 (0.67-0.83) versus 1.02 (0.85-1.41), $p < 0.05$). Measured cefazolin concentrations were best described by a two-compartment

population PK model with saturable protein binding. The covariate analysis showed that central volume of distribution increased linearly with body weight and that cefazolin distribution from the central to the subcutaneous compartment decreased with body weight in a non-linear manner. Based on the final covariate population PK model, Monte Carlo simulations were performed indicating that a dose of 2 g cefazolin given prior to incision will be sufficient to prevent wound infections with pathogens for which the minimal inhibitory concentration (MIC) is 1 mg/L for a duration of 240 minutes. In contrast, the probability of target attainment for morbidly obese versus non-obese patients for MIC values of 2 and 4 mg/L is reduced (Chapter 4, Table 3 and Figure 4).

In conclusion, this study showed that cefazolin distribution to the ISF of the subcutaneous adipose tissue is reduced in morbidly obese versus non-obese patients, that cefazolin tissue distribution reduces with increasing body weight and that dose adjustments are required in this patient group (see Appendix I).

Influence of morbid obesity and weight loss surgery on the pharmacokinetics of CYP3A substrate midazolam

According to the literature review in Chapter 2 decreased CYP3A mediated clearance in obese individuals may be expected. Therefore, in **Chapter 5** we aimed to study the pharmacokinetics of midazolam in morbidly obese patients versus non-obese healthy volunteers after semi-simultaneous oral and intravenous administration¹⁵. Midazolam is a widely applied drug for short-term and long-term sedation for procedures or at the intensive care unit. It is primarily metabolized by CYP3A into 1-OH-midazolam and as such considered a probe substrate for CYP3A activity¹⁶.

In a clinical study, 20 morbidly obese patients with a mean body weight of 144 kg (range 112–186 kg) and mean body mass index 47 kg/m² (range 40–68 kg/m²) participated in the study. All patients received a midazolam 7.5 mg oral and 5 mg intravenous dose separated by 159 ± 67 minutes. In addition, data from 12 healthy volunteers were available for a population pharmacokinetic (PK) analysis using NONMEM. In the final PK model, it was found that in morbidly obese patients the population mean clearance (relative standard error %) was similar (0.36 (4%) L/min), while oral bioavailability was higher in comparison to healthy volunteers (60% (13%) versus 28% (7%), $p < 0.001$). Furthermore, we found that central and peripheral volumes of distribution increased substantially with body weight (both $p < 0.001$).

In conclusion, in morbidly obese patients, systemic plasma clearance of midazolam is unchanged, while oral bioavailability is increased. Given the large increase in volumes of distribution, dose adaptations for intravenous midazolam should be considered (see Appendix I). Further research should elucidate the exact physiological changes at intestinal and hepatic level contributing to our observations of unchanged midazolam clearance and increased oral bioavailability in morbidly obese patients.

Besides the influence of morbid obesity on the pharmacokinetics on CYP3A substrate midazolam, the influence of bariatric surgery and its associated weight loss was evaluated. For his purpose, the patients from the study in Chapter 5 were invited to participate at a second study occasion one year after the bariatric procedure. The outcomes of these investigations are reported in **Chapter 6**¹⁷.

Of the 20 morbidly obese adult patients (144 ± 22 kg) who participated in the study of Chapter 5, 18 patients participated (mean loss of 45 ± 10 kg) one year after surgery. At both study occasions, patients received 7.5 mg oral and 5 mg intravenous midazolam separated by 160 ± 48 minutes. Using population pharmacokinetic modeling, it was found that, one year after bariatric surgery, systemic clearance of midazolam was higher (0.65 (7%) versus 0.39 (11%) L/min, $p < 0.01$, respectively). This increase in clearance after bariatric surgery could not be attributed to the decrease in body weight as the body weight model was inferior to the bariatric surgery model ($p < 0.05$). In addition, mean oral transit time was faster (23 (20%) versus 51 (15%) minutes, $p < 0.01$), while oral bioavailability was unchanged (0.54 (9%)). Central and peripheral volumes of distribution were overall lower in patients one year after bariatric surgery ($p < 0.05$).

Concluding, in this cohort study in morbidly obese patients undergoing bariatric surgery, systemic clearance (CL) was 1.7 times higher one year after bariatric surgery, which may potentially result from an increase in hepatic CYP3A activity per unit of liver weight. Although oral transit time was found to be faster, oral bioavailability (F) remained unchanged, which considering the increased systemic clearance (CL) implies an increase in the fraction escaping intestinal first pass metabolism (F_G).

Based on the results of Chapter 6, it was hypothesized that the midazolam fraction escaping gut wall metabolism (F_G) is increased in patients after bariatric surgery in comparison with morbidly obese patients before bariatric surgery. Knowledge on the exact influence of a bariatric procedure on hepatic and gut wall CYP3A activity, and therefore the fraction escaping hepatic metabolism (F_H) and F_G , may be of value for many other drugs, as approximately 30% of all clinically used drugs are metabolised via CYP3A¹⁸. Therefore, in **Chapter 7** we aimed to describe the pharmacokinetics of both midazolam and its CYP3A mediated metabolite 1-OH-midazolam in morbidly obese patients before and one year after bariatric surgery after both oral and intravenous administration¹⁹. A semi-physiologically based PK (Semi-PBPK) model taking into account gut wall and hepatic first pass metabolism was required for this analysis. The results of the model were used to explore to what extent these results may affect other CYP3A substrates.

Using a semi-PBPK model, it was found that for bariatric patients midazolam intrinsic hepatic clearance ($CL_{\text{int hepatic}}$) was 1.5 times higher ($p < 0.01$) in comparison to morbidly obese patients before surgery, resulting in a decrease in F_H of midazolam in patients after bariatric surgery. In contrast, intrinsic midazolam gut wall clearance ($CL_{\text{int gut wall}}$)

showed a trend towards lower values in bariatric patients, while for both patients groups values were considered low. As a result, F_G was close to one both for patients before and after for weight loss surgery, while especially the morbidly obese patient group exhibited large inter individual variability. Simulations of increased hepatic CYP3A abundance by 1.5 times showed a plasma clearance increase of 1.30-1.41 for low extraction ratio CYP3A substrates such as cyclosporine, alprazolam and triazolam using the SimCYP simulator²⁰. For the medium extraction ratio CYP3A substrate midazolam, this resulted in only a 1.22 increase in plasma clearance.

As this factor of 1.22 is lower than the factor of 1.7 identified in Chapter 6, the results of Chapter 7, in combination with the results from Chapter 5 and 6, have been summarized in Figure 1. In this figure, plasma clearance (CL_{plasma}) values and intrinsic blood clearance values at the level of the gut wall ($CL_{\text{int gut wall}}$) and liver ($CL_{\text{int hepatic}}$) are compared to values for healthy volunteers and bariatric patients from the perspective of the morbidly obese patient. From this figure, it may be concluded that the increase in midazolam plasma clearance after a bariatric surgery is not only due to normalization of previously reduced

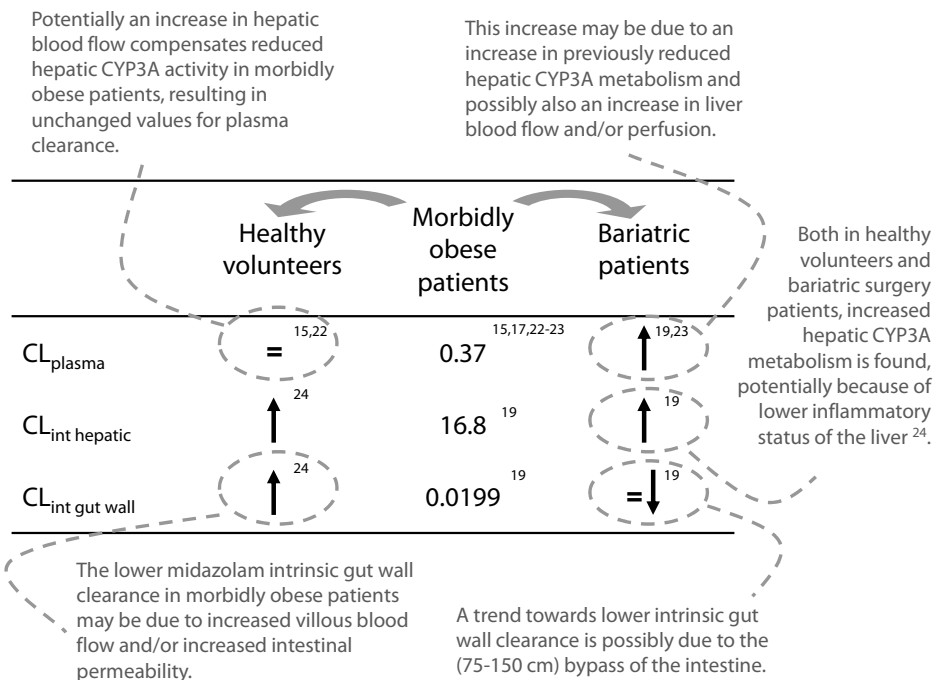


Figure 1 An overview of results on midazolam plasma clearance (CL_{plasma} (L/min)), intrinsic blood clearance at the level of the gut wall ($CL_{\text{int gut wall}}$ (L/min)) and intrinsic blood clearance at the level of the liver ($CL_{\text{int hepatic}}$ (L/min)) in morbidly obese patients in comparison to healthy (non-obese) volunteers and bariatric patients. The results are taken from Chapters 5, 6 and 7 and literature²²⁻²⁴. The grey arrows indicate the direction of comparison.

hepatic CYP3A activity (that is related to morbid obesity), but that at the same time another non-CYP3A related process is involved in an increase in midazolam plasma clearance (Chapter 6) ¹⁷. This other process may be an increase in hepatic blood flow (Q_H) or hepatic perfusion ²¹. An increase in Q_H in morbidly obese patients in comparison with healthy volunteers may also explain the similar midazolam plasma clearance in morbidly obese patients in comparison with healthy volunteers, despite reduced hepatic CYP3A activity related to morbid obesity (Chapter 5 and Figure 1) ¹⁵.

In conclusion, a semi-PBPK model was identified that adequately described midazolam and CYP3A mediated 1-OH-midazolam concentrations after both oral and intravenous administration. Using this model it was found that in patients one year after bariatric surgery CYP3A hepatic intrinsic metabolizing capacity is increased in comparison to morbidly obese patients before bariatric surgery. However, CYP3A mediated gut wall intrinsic clearance shows a trend towards lower values in bariatric patients, probably as a result of the 75-150 cm bypass of the initial part of the small intestine.

PERSPECTIVES

How to get to evidence-based dosing in morbidly obese or bariatric patients

In this thesis, two drugs have been studied using well-designed clinical trials resulting in evidence-based dosing recommendations for morbidly obese and bariatric patients (see **Appendix I**). However, to establish evidence-based dosing for morbidly obese patients before and after weight loss surgery for every clinically used drug separately, as we did in this thesis, will be time and cost consuming. Hence, an intriguing question is how we can accelerate and facilitate this process. One way would be to evaluate whether the PK models that were developed for specific drugs as we did in this thesis may contain information that can be considered system specific information for this population. Such system specific information may potentially be used for predictions for other (unstudied) drugs.

This concept has been explored earlier for both UGT2B7 glucuronidation and renal elimination in neonates and children ²⁵⁻³¹. These studies showed that the covariate function for a population characteristic (e.g. body weight) derived for one so called model drug was predictive for the changes in clearance of another drug cleared through the same pathway. For changes in clearance of the UGT2B7 substrate zidovudine in young infants, the same influence of body weight was found as for the UGT2B7 substrate morphine and potentially other substrates of UGT2B7 ^{28-29, 32}. Also, for drugs cleared via glomerular filtration, it was found that the covariate model for amikacin clearance in the heterogeneous group of preterm and term neonates was able to describe the clearance

of other glomerularly filtrated drugs such as netilmicin, vancomycin, tobramycin, and gentamycin across this population^{27,30}.

For obese patients, we tended to apply a similar approach for which the literature overview of Chapter 2 was written. In fact, this overview showed coherent trends of the influence of obesity on the different classes of clearance pathway. However, the observed trends should be prospectively evaluated before they can be used to predict the impact of obesity on an unstudied drug. This particularly applies to morbidly obese individuals, because the studies in the review predominantly included overweight and obese patients as opposed to morbidly obese patients.

In this thesis, we found for cefazolin (Chapter 4) which is mainly eliminated by glomerular filtration (GFR) and active tubular excretion¹¹, that there was no influence of body weight on clearance. Also for the CYP3A substrate midazolam (Chapter 5), no effect of morbid obesity on clearance was found in comparison to healthy volunteers. A crucial question in this respect is whether these results can be applied to other renally cleared and CYP3A metabolized drugs, respectively. The review in Chapter 2 shows that of the ten studies involved in glomerularly filtrated drugs, six studies show a significant increase in clearance and four studies show no difference, indicating that the influence of morbid obesity on glomerular filtration may not be straightforward to predict. This is in line with studies reporting that both GFR hyperfiltration and impairment may be present in the general morbidly obese patient population, with impairment reported particularly upon a prolonged state of morbid obesity³³⁻³⁵. As such it seems that our findings for cefazolin are in agreement with literature as we report unchanged cefazolin clearance in a morbidly obese patient group that was relatively young (mean age 40 years) with normal creatinine concentrations at inclusion of the study.

The lack of difference in clearance of the CYP3A substrate midazolam between morbidly obese patients and non-obese volunteers (Chapter 5) seems to be in contrast with the results of the review of Chapter 2 in which several studies showed significantly lower oral clearance (CL/F) of CYP3A mediated drugs. However, as we found a higher midazolam oral bioavailability (F) in morbidly obese patients in comparison to healthy volunteers (60 versus 28%, respectively), indeed a lower CL/F for midazolam in morbidly obese patients can be reported. Using a more sophisticated approach in Chapter 7 (using semi-PBPK model for midazolam and 1-OH midazolam), a lower CYP3A metabolizing capacity of the liver was found in morbidly obese patients in comparison to non-obese healthy volunteers from the literature. This reduced CYP3A activity in morbidly obese patients is in good agreement with results of *in vitro* and animal studies showing reduced CYP3A protein expression and activity³⁶⁻³⁹. Despite this reduced CYP3A activity in the liver, similar plasma clearance values for morbidly obese and non-obese individuals were found (Figure 1, Chapter 5&7). As a consequence, it is hypothesized that another process (e.g. liver blood flow, liver perfusion and/or size of the liver) compensates for this

reduction in hepatic CYP3A metabolizing capacity. It therefore seems that for the success of between drug extrapolation per pathway information on obesity-related changes in liver blood flow, size and perfusion are needed. Future studies should therefore not only focus on the quantification of the influence of obesity on drug different metabolism and elimination pathways using model drugs, but also on the quantification of changes in liver blood flow, size and liver perfusion to be able to make better informed predictions on how plasma clearance of an unstudied drug will change with obesity. In this respect, also the hepatic extraction ratio of a drug may play a role ⁴⁰, as plasma clearance of low extraction ratio drugs are more sensitive to changes in metabolizing activity of the involved enzyme system and high extraction ratio and drugs are more sensitive to changes in the blood flow (Chapter 7, Figure 5).

The studies reported in this thesis show that particularly the volume of distribution of the two drugs studied are impacted by morbid obesity. Jain *et al.* showed that the influence of obesity on hydrophilic drugs may be predicted based on the Log P value, while for (highly) lipophilic drugs no such trends can be observed ⁴¹. As the change in distribution volume for the two drugs studied in this thesis were responsible for the proposed dose adaptations for morbidly obese patients (Appendix I), concepts to predict the influence of obesity on volume of distribution are needed. Volume of distribution is determined by drug characteristics, including protein binding, transporter dependency, the ability to cross tissue membranes, binding within blood and tissues and partitioning into fat ⁴⁰. In addition, volume of distribution is determined by systemic properties of which in particular blood volume, adipose tissue volume, cardiac output and tissue perfusion are impacted by obesity ^{21, 42-43}. Theoretically, when the influence of obesity on the systemic parameters governing volume of distribution are known and drug characteristics are known, it should be possible to predict the change in volume of distribution for a specific drug in a specific obese individual. Huisinga *et al.* have proposed such a model for estimating volume of distribution at steady state (V_{ss}) based on the concept that adipose tissue volume equals total body weight (TBW) minus lean body weight (LBW) ⁴⁴:

$$V_{ss} = V_{s,ref} \times \left((1-R) \times \frac{LBW}{LBW_{ref}} + R \times \frac{TBW - LBW}{TBW_{ref} - LBW_{ref}} \right)$$

in which LBW is the lean body weight (estimated by the formula of Janmahasatian *et al.* ⁴⁵), '*ref*' indicates the reference individual (a non-obese healthy volunteer age 20-50 years) and R denotes the adipose-to-total volume of distribution ratio of the reference individual which can be estimated from clinical data ⁴⁴. A drawback of this model seems that the value R comprises all drug characteristics and needs to be determined for each drug individually. Therefore, the applicability of this model is unclear at this point and

should be subject of future studies. Alternatively, physiological models in which drug characteristics regarding drug distribution can be defined (e.g. log D, ionization at pH 7.4, protein binding, tissue partition coefficients, etc.) seem more promising as opposed to a single drug parameter. Currently, several software packages of such physiologically based pharmacokinetic (PBPK) models are available, including SimCYP®^{20, 46}, which deserve to be explored for their predictive value to estimate volume of distribution in (morbidly) obese individuals.

The PBPK software package SimCYP® also proved capable of mimicking observed clearance values in (morbidly) obese patients for 6 out of 8 compounds in 60-100% of the simulations using the 'obese' and 'morbidly obese' population of this program⁴⁷. However, for 2 compounds (phenytoin and clorzoxazone) clearance predictions were in good agreement for only 20% of the simulations⁴⁷, implying that these models need further information, in particular as the exact influence of (morbid) obesity on some physiologic parameters (e.g. hepatic blood flow and perfusion) remains unclear. While this approach seems very promising for predicting the pharmacokinetics of unstudied drugs in (morbidly) obese patients, in our opinion close collaboration with groups performing clinical trials in morbidly obese patients are important to further inform and improve the predictability of these PBPK models.

These concepts for predicting pharmacokinetics in (morbidly) obese patients may also be applied for patients after weight loss (bariatric) surgery. For this population, the type of bariatric surgery, time after bariatric surgery and decrease in body weight should be considered. Darwich *et al.* have aimed to predict the disposition of drugs in patients after different types of bariatric surgeries using an adjusted advanced drug absorption and metabolism (ADAM) model combined with a PBPK model⁴⁸. On the basis of this model, the authors were able to adequately predict the trends in oral drug exposure of atorvastatin and cyclosporine (CYP3A substrates) following a Roux-and Y-gastric bypass surgery⁴⁸. However, this model did not yet include a recovery of hepatic CYP3A metabolizing capacity as indicated by the Chapters 6 and 7. This further underlines the need to perform clinical trials on (model) drugs to inform PBPK models which can then be applied to predict the influence of morbid obesity and bariatric surgery on unstudied drugs. In addition to the recovery of intrinsic hepatic CYP3A metabolizing capacity, knowledge on the change in physiologic parameters due to the reduction in body weight (e.g. cardiac output, liver perfusion, adipose tissue volume, etc.) is needed to further enhance the applicability and predictability of such a PBPK model for patients after weight loss surgery.

In conclusion, on the basis of these concepts the process of getting to evidence-based dosing recommendations for all clinically used drugs for the morbidly obese and bariatric surgery patient population may be accelerated and as such it seems that further study on these concepts are justified.

Tips and tricks for the design and analysis of studies in morbidly obese and bariatric surgery individuals

While additional clinical trials in morbidly obese before and after weight loss surgery are evidently needed, this section aims to emphasize on methods and techniques for future clinical research that ultimately aim to guide dosing in morbidly obese patients before and after weight loss surgery.

First, for future studies it is recommended to measure and quantify the clinical effects (pharmacodynamics, PD) in addition to pharmacokinetics (PK), as in the end it is the clinical effect that will determine the optimal dose for the individual morbidly obese or bariatric patient. In this thesis, the pharmacodynamics of both the drugs that were studied for the PK, have been measured. For cefazolin, the clinical desired effect is the prevention of surgical wound infections. Due to the nature of this clinical endpoint, large trials will be needed to measure the effect of a single cefazolin dose. Therefore, we have evaluated the cefazolin concentrations which are expected to correlate most closely with its antibacterial effect, i.e. the subcutaneous tissue ISF concentrations, in addition to unbound and total cefazolin plasma concentrations (Chapter 4). To date, clinical microdialysis is the only sampling technique that allows for measurement of free, active concentrations in virtually any tissue such as ISF⁴⁹⁻⁵⁰. In addition, clinical microdialysis has been shown to be a safe, reproducible, and an ethically acceptable technique for studying tissue drug distribution in human⁵¹⁻⁵². Alternative methods for measuring tissue concentration include tissue biopsies or blister fluid techniques⁵³⁻⁵⁴. A draw back of these alternative methods is that they do not easily allow for continuous measurements. Furthermore, tissue biopsies are homogenized which prevents the measurement of inter- and intra- cellular concentration separately, while for antimicrobial agents only the inter cellular concentration is of interest, as this fraction is expected to exert an antimicrobial effect. In addition, the blister fluid technique may be quite painful for each blister made for each measurement⁵⁵. In contrast, in case of clinical microdialysis, the insertion of the microdialysis membrane may be considered moderately painful as well, but is only performed once at insertion. After insertion no pain was experienced until the end of the study. So, in the study of Chapter 4, clinical microdialysis facilitated insight into cefazolin target site penetration in morbidly obese patients. It is emphasized that these measurements in the ISF proved crucial for conclusions regarding cefazolin dosing in this patient population as plasma concentrations proved relatively similar while subcutaneous tissue distribution and concentrations were largely reduced in morbidly obese patients in comparison to non-obese patients^{12,14}.

For the midazolam study of Chapter 6, sedation scores were recorded from midazolam oral dose administration until 160 ± 48 minutes after dosing. In Figure 2, the Richmond Agitation and Sedation Scale (RASS) scores are shown for morbidly obese patients before (occasion 1) and one year after bariatric surgery (occasion 2). For occasion 1, 10 out

of 19 morbidly obese patients were sedated to some extent (score -1 and/or -2), while 9 out of 19 patients showed no sign of sedation (RASS = 0). One year later, 16 out of 18 patients showed some level of sedation (score -1 to -4), while only 2 patients showed no level of sedation. Moreover, sedation was deeper after bariatric surgery (maximum of -4 reported in 4 patients) compared to before bariatric surgery (maximum of -2 in 2 patients) and seemed to occur slightly faster. The sedation levels versus times profiles suggest that the midazolam concentration-time profile is indicative of its sedative effect in morbidly obese patients before and after bariatric (Figure 2). However, it should be noted that the less deep sedation levels observed in morbidly obese patients before bariatric surgery may also be the result of anxiety related to the surgical procedure that may be experienced by these patients while one year after surgery no surgical procedure

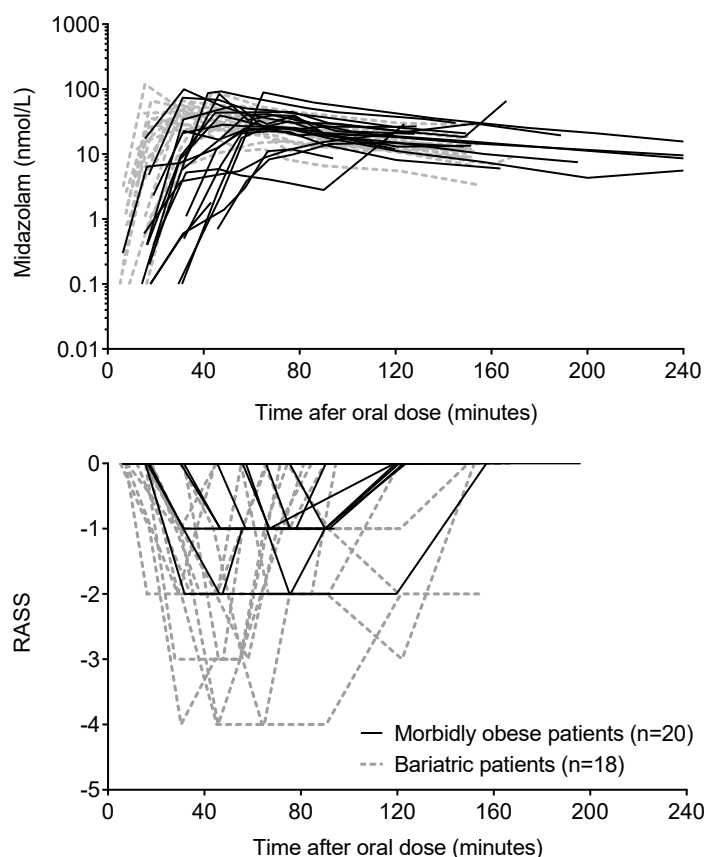


Figure 2 Midazolam concentration versus time (upper panel) and Richmond Agitation Sedation Scale (RASS) scores over time (lower panel) after a 7.5 mg oral midazolam dose in 20 morbidly obese patients before (black lines) and 18 patients one year after bariatric surgery (grey dotted lines) from the study described in Chapter 6.

was scheduled. In conclusion, for both of the drugs studied in this thesis, drug effects have been measured in addition to pharmacokinetic profiles, which may provide a more profound basis for dose recommendations in morbidly obese and bariatric surgery patients.

Second, it is recommended to evaluate the pharmacokinetics after both oral and intravenous administration in order to estimate the influence of morbid obesity or bariatric surgery for each PK parameter separately (i.e. oral bioavailability (F), clearance (CL) and volume of distribution (V)). In studies in which the drug is administered orally, only the apparent clearance (CL/F) and volume of distribution (V/F) can be determined, while studies on intravenous administered drug result in estimates of CL and V and not of F. In particular because the majority of pharmacotherapy is given orally, knowledge on how F or CL are each impacted by morbid obesity or bariatric surgery is essential for the extrapolation of the results to other drugs as described in 8.2.1.. For this reason in Chapter 5 and 6, a semi-simultaneous oral-intravenous dosing design was applied. Earlier, it had been shown that this semi-simultaneous dosing design method is a reliable and accurate for estimating oral bioavailability (F) and systemic clearance (CL) in a single person, on a single occasion⁵⁶⁻⁵⁹. Alternatively, the stable isotope method for determining oral bioavailability in a single person on a single occasion may be applied⁶⁰. However, the preparation of the labeled drug and the determination of the labeled drug in the samples may be expensive and labor intensive. In conclusion, a semi-simultaneous dosing design allows for separate estimation of both CL and F (Chapter 5 and 6) and therefore also of hepatic and gut wall CYP3A mediated metabolism (Chapter 7).

Third, when designing a clinical trial the choice of control or reference group determines the type of results and conclusions that can be drawn from the trial. In this thesis, different types of control groups have been used: non-obese (never been obese) patients undergoing a Toupet fundoplication laparoscopic procedure (Chapter 4), healthy (never been obese) volunteers (Chapter 5) and the same patients (who were morbidly obese at the time) one after year a bariatric surgery (Chapters 6 and 7). Besides the specific advantages and drawbacks of each type of control group, it can be expected that comparing two groups does not allow for estimation of a continuous covariate function, but rather a binary function ('obese/non-obese'), to describe the influence of overweight or obesity, while in fact the overweight itself (normal weight to super obese) may be expected to be a continuous parameter. For instance, when analysing midazolam PK in morbidly obese patients versus healthy volunteers (Chapter 5) a large difference in oral bioavailability (F) was found, which was defined by a binary covariate, 'obese/non-obese'. Whether and how midazolam F changes with increasing degree of overweight or obesity remains unclear as no midazolam concentrations in individuals in between healthy volunteer and morbidly obese were available. Following this, it can be expected that the predictability of midazolam oral bioavailability by this particular

lumped into an empirical three compartment population PK model. In addition, further simplification of the model was performed assuming quasi steady state approximation as outlined in Supplement 2 of Chapter 7⁶⁵. This model simplification allowed omitting setting values for the organ volumes of the gut wall, liver and portal vein, while giving approximately the same model prediction (Supplement 2 of Chapter 7). Ultimately, this model only requires knowledge on hepatic and villous blood flow and protein binding of the population studied to estimate midazolam clearance at the level of the gut wall and liver. For future projects, semi-PBPK modeling may be applied to estimate intrinsic metabolizing capacity in special patient populations providing insight at what level (blood flow, protein binding, intrinsic metabolic capacity, etc.) drug clearance is impacted by obesity or bariatric surgery and thus enhancing the extrapolation potential of the results.

Finally, in future trials physiological parameters should be collected in order to understand the observed changes in the pharmacokinetics and to enhance the applicability of PBPK models. The conversion of the influence of obesity on CYP3A activity in the liver to plasma clearance of midazolam and other CYP3A substrates as reported in the Chapters 6 and 7, show the relevance of obesity related changes in the physiology of the obese individual. For instance, hepatic blood flow is an important physiological parameter involved in the clearance of many medium and high hepatic extraction rate drugs (e.g. midazolam). Yet, it is still unclear whether and how hepatic blood flow changes with obesity and bariatric surgery. It has been reported that cardiac output increases with (morbid) obesity⁶⁶ and that the percentage of cardiac output going to the hepatic blood flow is similar to (predicted) normal weight individuals²¹. Furthermore, studies on the pharmacokinetics of propofol, a high extraction ratio drug and therefore considered a marker of hepatic blood flow⁶⁷, indicate a 0.75 allometric increase in hepatic blood flow with body weight⁶⁸⁻⁶⁹. In contrast, a study in animals shows that hepatic blood flow and perfusion (hepatic microcirculation) reduce with the degree of fatty infiltration in the liver, a condition which is highly associated with (morbid) obesity⁷⁰⁻⁷¹. Moreover, data on hepatic blood flow values in bariatric patients are non existent. This lack of information prevents a full understanding of the change in midazolam clearance observed in bariatric patients in comparison to morbidly obese patients (Chapter 7). Therefore, we recommend that in future clinical trials in morbidly obese and/or bariatric patients physiological parameters should be measured in order to enlarge the predictability and extrapolation potential of the results from clinical trials to other (unstudied) drugs. Lastly, it should be noted that the collection of such physiologic parameters may not be easy, as not all methods (e.g. Flo Trac/Vigileo™ for measuring cardiac output) have been validated in the (morbidly) obese population and may therefore be inappropriate.

In conclusion, the most efficient way towards evidence-based dosing in morbidly obese and bariatric patients is to perform optimally designed clinical trials in morbidly obese and bariatric patients, to investigate how system specific properties (e.g. CYP3A activity) can be inferred from these trials and to evaluate whether these system specific properties may predict the impact of obesity and weight loss surgery on other (unstudied) drugs. The clinical studies should be designed to include the complete range overweight to super obese patients (BMI 25 – 100+ kg/m²) and in case of bariatric surgery, various types of bariatric surgery and periods after the bariatric procedure. Within these clinical trials one should aim to collect data on concentration-time profiles (pharmacokinetic data, PK), clinical effects (pharmacodynamic data, PD) as well as physiological parameters (e.g. blood flows, protein binding, etc.). Finally, PBPK(-PD) models which allow for the integration of pharmacokinetic and physiologic parameters and possibly also pharmacodynamic parameters for the obese and bariatric patients population should be further developed to improve prediction of the impact of obesity and bariatric surgery on unstudied drugs. With this thesis we hope to have contributed to this relevant topic.

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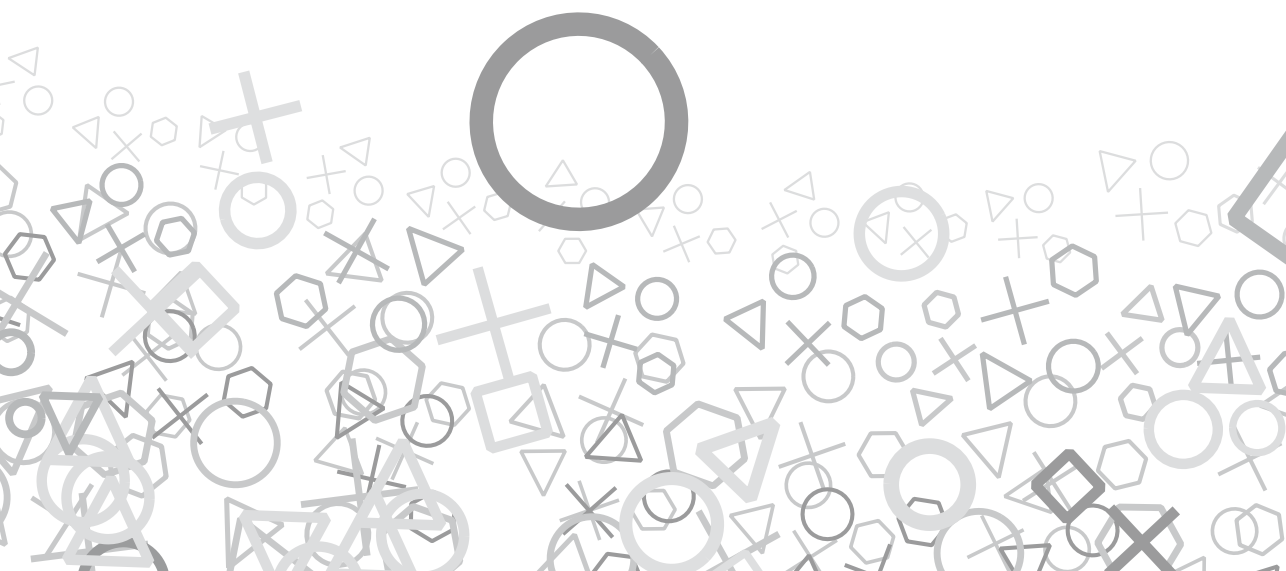
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CHAPTER 9

Nederlandse Samenvatting/Dutch Summary



CONCEPTEN EN TOEPASSINGEN VOOR HET DOSEREN VAN GENEESMIDDELEN OP BASIS VAN WETENSCHAPPELIJKE ONDERBOUWING IN MORBIDE OBESE PATIËNTEN VÓÓR EN NA EEN MAAGVERKLEININGSOPERATIE

ACHTERGROND EN INTRODUCTIE

In Nederland en wereldwijd is er een toename van mensen met obesitas of morbide obesitas (zeer ernstig overgewicht) ¹⁻². Obesitas wordt gedefinieerd als een body mass index (BMI) groter dan 30 kg/m² en bij een BMI groter dan 40 kg/m² spreekt men van morbide obesitas.

Obesitas en morbide obesitas kunnen veranderingen teweeg brengen in de samenstelling en functies van het lichaam. Deze veranderingen kunnen vervolgens van invloed zijn op twee processen die bepalend zijn voor de werking van geneesmiddelen: de farmacokinetiek (PK) en de farmacodynamiek (PD). De farmacokinetiek beschrijft de absorptie (opname), verdeling, metabolisme (afbraak) en uitscheiding van het geneesmiddel en daarmee het verloop van de geneesmiddelconcentratie in het lichaam over de tijd. De farmacodynamiek beschrijft het effect en werking van het geneesmiddel op het lichaam.

Doordat (morbide) obesitas de blootstelling (farmacokinetiek) en effecten (farmacodynamiek) van geneesmiddelen kunnen veranderen, wordt er mogelijk teveel of te weinig van een bepaald geneesmiddel toegediend wanneer de standaard dosering (voor niet-obese patiënten) wordt gebruikt. Om deze reden en het feit dat mensen met (morbide) obesitas een verhoogd risico hebben op het ontwikkelen van verschillende gerelateerde aandoeningen zoals kanker, diabetes type 2 en cardiovasculaire aandoeningen ³, zijn er voor obese en morbide obese patiënten doseeradviezen nodig die gebaseerd zijn op wetenschappelijk onderbouwing.

Op dit moment is de invloed van morbide obesitas op de absorptie, verdeling, metabolisme en uitscheiding (farmacokinetiek) van geneesmiddelen grotendeels onbekend. De studies die reeds zijn uitgevoerd en gepubliceerd, vonden met name plaats in mensen met overgewicht (BMI 25-30 kg/m²) en obese patiënten (BMI 30-40 kg/m²), waardoor er een sterke behoefte is aan farmacokinetiek onderzoek in morbide obese patiënten. Uiteindelijk zal ook de invloed van morbide obesitas op de farmacokinetiek-farmacodynamiek (PK-PD) relatie bestudeerd moeten worden om de juiste geneesmiddeldosering in deze populatie te bepalen.

Door de toename van morbide obesitas, neemt ook het aantal bariatrische (gewichtsheterducerende) operaties toe ⁴. Een bariatrische operatie, of ook wel een maagverkleining genoemd, wordt beschouwd als de meest effectieve behandeling van morbide obesi-

tas⁵⁻⁶. Deze operatieve ingreep resulteert onder andere in langdurig gewichtverlies, het verdwijnen van diabetes type 2 en langere overleving⁶⁻⁷. Er worden veel verschillende typen maagverkleiningsoperatie uitgevoerd, waarvan de Roux- and Y gastric bypass en de sleeve gastrectomy de meest voorkomende zijn (circa 75% wereldwijd⁴). Bij een Roux- and Y gastric bypass wordt de maag tot de grootte van ongeveer een ei gereduceerd en het eerste deel van de dunne darm (75-150 cm) wordt omgelegd. Bij een gastric sleeve operatie wordt de maag tot een buis gereduceerd, maar vindt er geen omlegging van de darm plaats⁸⁻⁹. Voor artsen en apothekers roept deze steeds groter wordende groep bariatrische patiënten veel vragen en onduidelijkheid op omtrent veilige en effectieve geneesmiddeltherapie, aangezien deze typen operatie van sterke invloed kunnen zijn op de farmacokinetiek van een geneesmiddelen. Immers, de operaties hebben zowel een aanpassing van het maag-darmkanaal als een groot gewichtverlies tot gevolg. Gemiddeld verliest iemand na een maagverkleiningsoperatie 32% van zijn totale lichaamsgewicht binnen 0,5-2 jaar na de ingreep⁶. Om deze redenen is er een sterke vraag naar informatie over de veranderingen in de farmacokinetiek en –dynamiek van geneesmiddelen en naar wetenschappelijk onderbouwde doseeradviezen bij patiënten met een maagverkleining.

Als een eerste stap in de richting van wetenschappelijk onderbouwde doseeradviezen voor mensen met (morbide) obesitas, wordt in **Hoofdstuk 2** van dit proefschrift een overzicht gepresenteerd van alle gerapporteerde farmacokinetiek studies in zowel (morbide) obese als niet-obese individuen. Deze studies zijn gesorteerd per afbraak (metabole) of eliminatie route van het onderzochte geneesmiddel¹⁰. Dit overzicht laat zien dat de invloed van obesitas op de klaring (uitscheidingssnelheid, CL) van een geneesmiddel, afhangt van de belangrijkste afbraak of eliminatie route die betrokken is bij de uitscheiding van dat geneesmiddel. Geneesmiddelen die bijvoorbeeld via het enzym systeem Cytochroom P450 3A (CYP3A) worden gemetaboliseerd hebben een lagere orale klaring (CL/F) in obese individuen. Echter, geneesmiddelen die bijvoorbeeld door uridine diphosfaat glucuronosyltransferase (UGT), glomerulaire filtratie of het CYP2E1 systeem afgebroken of uitgescheiden worden hebben juist een hogere geneesmiddelklaring in obese individuen dan in niet- obese individuen.

In **Hoofdstuk 3** wordt een overzicht gegeven van de invloed van obesitas op de orale absorptie (opname), verdeling, en klaring (afbraak en/of uitscheiding) van een geneesmiddel en worden aanwijzingen voor toekomstig onderzoek op dit gebied samengevat¹¹. Dit hoofdstuk wijst op het feit dat het verdelingsvolume van een geneesmiddel zeer sterk beïnvloed kan worden door (morbide) obesitas, waarbij de grootte en richting van verandering met toenemende obesitas niet goed te voorspellen is op basis van alleen de lipofiliciteit (mate van vetoplosbaarheid) van een geneesmiddel. In vergelijking met de invloed van (morbide) obesitas op het verdelingsvolume, lijkt het

erop dat de invloed van (morbide) obesitas op de klaring van een geneesmiddel kleiner is en voorspelbaar per metabole of eliminatie route (zie Hoofdstuk 2). Tenslotte wordt uit Hoofdstuk 3 duidelijk dat er weinig bekend is over de invloed van (morbide) obesitas op de orale absorptie snelheid (K_a) en de biologische beschikbaarheid (F) van geneesmiddelen, terwijl een beperkt aantal studies laat zien dat de orale absorptie mogelijk veranderd is.

Gezien het tekort aan kennis over orale absorptie, verdeling en klaring van geneesmiddelen in morbide obese patiënten en patiënten na een bariatrische operatie en het gebrek aan wetenschappelijk onderbouwde doseeradviezen voor deze patiëntengroepen, hebben wij in dit proefschrift twee verschillende geneesmiddelen bestudeerd in deze patiëntengroepen. Ten eerste hebben we de onderhuidse weefselpenetratie van cefazoline in morbide obese patiënten tijdens een bariatrische ingreep onderzocht. Ten tweede hebben we de invloed van zowel morbide obesitas als een bariatrische ingreep op de farmacokinetiek van het geneesmiddel midazolam, dat via het CYP3A enzym systeem wordt gemetaboliseerd, onderzocht na orale en intraveneuze toediening.

De geneesmiddelconcentratie-tijd gegevens uit deze onderzoeken werden geanalyseerd met populatie farmacokinetiek modellen (met behulp van het software programma NONMEM¹²⁾¹³. Met deze analyse methode kunnen op basis van de gezamenlijke concentratie-tijd gegevens de farmacokinetische parameters (K_a , F, V, CL) geschat worden. Daarbij kan een inschatting van de interindividuele variabiliteit per parameter gemaakt worden, waardoor de invloed van covariaten, zoals bijvoorbeeld lichaamsgewicht, mate van obesitas, etc., op elk van de farmacokinetiek parameters bepaald kunnen worden.

INVLOED VAN MORBIDE OBESITAS OP DE FARMACOKINETIEK VAN CEFAZOLINE

Het geneesmiddel cefazoline is een eerste generatie cefalosporine antibioticum dat wordt gebruikt bij veel verschillende operaties, waaronder bariatrische ingrepen, om ontsteking (infectie) van operatiewonden te voorkomen¹⁴. Cefazoline wordt uitgescheiden door glomerulaire filtratie en actieve tubulaire excretie in de nieren¹⁵.

Onderzoek toont aan dat morbide obese patiënten meer wondinfecties krijgen na een operatie, terwijl de cefazoline concentraties in het bloedplasma adequate spiegels lijken te bereiken¹⁶⁻¹⁷. Of cefazoline ook adequate spiegels bereikt op de plek van werking is onduidelijk. De plek van werking is in dit geval de interstitiële vloeistof (ISF) van het onderhuidse vetweefsel rondom de operatiewondjes (buik). Om dit in kaart te brengen, stelden we ons in **Hoofdstuk 4** als doel om ongebonden cefazoline concentraties in het ISF van het onderhuidse vetweefsel van morbide obese patiënten en niet-obese

patiënten te meten en te vergelijken. De resultaten werden gebruikt om de invloed van morbide obesitas op de farmacokinetiek van cefazoline in het onderhuidse vetweefsel te kwantificeren, waarbij rekening werd gehouden met de eiwitbinding van cefazoline¹⁸.

Na intraveneuze toediening van 2 gram cefazoline werden bloedplasma monsters verzameld en werden totaal en ongebonden cefazoline concentraties gemeten in 9 morbide obese patiënten (141 ± 22 kg, 107-175 kg) en 7 niet-obese patiënten (86 ± 13 kg, 72-109 kg). Met behulp van klinische microdialyse werd ongebonden cefazoline in het ISF van het onderhuidse vetweefsel van de buik verzameld tot 4 uur na de intraveneuze toediening van cefazoline. Er werd gevonden dat de onderhuidse weefselpenetratie van ongebonden cefazoline, aangeduid als de ratio van de oppervlakte onder de ongebonden concentratie-tijd curven (AUCs), $fAUC_{\text{weefsel}}/fAUC_{\text{plasma}}$ lager was in morbide obese patiënten in vergelijking met niet-obese patiënten (0.70 (0.67-0.83) versus 1.02 (0.85-1.41), $p < 0.05$).

De cefazoline concentraties in plasma en onderhuids weefsel over de tijd werden het best beschreven met behulp van een twee-compartimenten populatie farmacokinetiek model met verzadigbare eiwitbinding. De analyse van de invloed van covariaten wees uit dat het centrale verdelingsvolume lineair toenam met lichaamsgewicht en dat de cefazoline verdeling van het centrale naar het onderhuidse weefsel afnam met toenemend lichaamsgewicht op een non-lineaire wijze. Op basis van dit populatie farmacokinetiek model werden Monte Carlo simulaties uitgevoerd. Deze simulaties lieten zien dat een cefazoline dosis van 2 gram (toegediend vóór de operatie) tot en met 240 minuten na toediening voldoende is om ontsteking van operatiewondjes door pathogenen, voor welke een minimale inhibitoire concentratie (MIC) van 1 mg/L gelden (Nederland), te voorkomen. Het percentage morbide obese patiënten die gedurende 240 minuten onderhuidse ongebonden cefazoline concentraties boven hogere MIC waarden (bijvoorbeeld 2 of 4 mg/L) halen, nam sterk af in vergelijking met niet-obese patiënten (Tabel 3 en Figuur 4 van Hoofdstuk 4).

Op basis van deze studie kan worden geconcludeerd dat de verdeling van cefazoline naar het onderhuidse vetweefsel in morbide obese patiënten is afgenomen in vergelijking met niet-obese patiënten, dat de weefselpenetratie van cefazoline afneemt met toenemend gewicht en dat er aanpassingen in de dosering nodig zijn voor morbide obese patiënten (zie het doseeradvies voor cefazoline in Appendix I).

INVLOED VAN MORBIDE OBESITAS EN EEN MAAGVERKLEININGSOPERATIE OP DE FARMACOKINETIEK VAN HET CYP3A SUBSTRAAT MIDAZOLAM

Het literatuuroverzicht van Hoofdstuk 2 geeft aan dat CYP3A gemedieerde klaring mogelijk lager is in individuen met (morbide) obesitas. Om dit nader te onderzoeken,

had **Hoofdstuk 5** tot doel om de farmacokinetiek van midazolam in morbide obese patiënten versus niet-obese gezonde vrijwilligers na orale en intraveneuze toediening te onderzoeken ¹⁹.

Midazolam is een veelgebruikt geneesmiddel voor sedatie bij korte ingrepen of op de intensive care afdeling. Het wordt voor het overgrote deel door het CYP3A enzym in 1-OH-midazolam omgezet en wordt om deze reden als 'probe substraat' voor de activiteit van het CYP3A enzym systeem beschouwd ²⁰. Het CYP3A enzym bevindt zich zowel in de lever als in de darmwand waardoor het bij zowel de klaring (via de lever) als de orale biologische beschikbaarheid (via de darmwand) van midazolam betrokken is.

In deze klinische studie namen 20 morbide obese patiënten met een gemiddeld lichaamsgewicht van 144 kg (minimum - maximum, 112–186 kg) en een gemiddeld BMI van 47 kg/m² (minimum - maximum, 40–68 kg/m²) deel. Alle patiënten kregen 7,5 mg orale midazolam dosis en een 5 mg intraveneuze dosis toegediend met een interval van 159 ± 67 minuten. Daarbij was er data van 12 gezonde vrijwilligers beschikbaar voor een gezamenlijke populatie farmacokinetiek analyse met behulp van NONMEM. Het uiteindelijke populatie farmacokinetiek model liet zien dat er geen verschil is in de klaring (CL) van midazolam in morbide obese patiënten versus gezonde vrijwilligers (0.36 (4 %) L/min), terwijl de orale biologische beschikbaarheid (F) hoger was in morbide obese patiënten in vergelijking met gezonde vrijwilligers (60 % (13 %) versus 28 % (7 %), p<0.001). Verder toonde deze studie aan dat de centrale en perifere verdelingsvolumes van midazolam substantieel toenamen met lichaamsgewicht (beide p<0.001).

Op basis van deze resultaten, kan worden geconcludeerd dat midazolam klaring onveranderd is in morbide obese patiënten, terwijl de orale biologische beschikbaarheid is verhoogd. Vanwege de grote toename van het verdelingsvolume van midazolam in morbide obese patiënten, moet er rekening worden gehouden met een aanpassing van de intraveneuze toediening van midazolam (zie het doseeradvies in Appendix I). Toekomstig onderzoek moet aantonen welke fysiologische veranderingen in de lever en darm bijdragen aan onze observaties ten aanzien van, respectievelijk, de onveranderde klaring en verhoogde biologische beschikbaarheid van CYP3A substraat midazolam in morbide obese patiënten.

Naast de invloed van morbide obesitas op de farmacokinetiek van het CYP3A substraat midazolam, is de invloed van een bariatrische operatie, en het daarmee gepaard gaande verlies in lichaamsgewicht, onderzocht. Voor dit doel zijn de morbide obese patiënten uit de studie van Hoofdstuk 5 één jaar nu hun bariatrische ingreep uitgenodigd om deel te nemen aan het tweede deel van het onderzoek. De uitkomsten van dit tweede deel zijn gerapporteerd in **Hoofdstuk 6** ²¹.

Van de 20 morbide obese patiënten (144 ± 22 kg), die deelnamen aan de studie in Hoofdstuk 5, namen 18 patiënten (gemiddeld gewichtsverlies van 45 ± 10 kg) opnieuw

deel aan het onderzoek één jaar na de operatie. De patiënten kregen opnieuw een 7,5 mg tablet midazolam en een 5 mg intraveneuze dosis toegediend met een interval van gemiddeld 160 ± 48 minuten. Met behulp van populatie farmacokinetiek modelleren werd gevonden dat de plasmaklaring (CL) van midazolam een jaar naar de bariatrische operatie, hoger was in vergelijking met ervoor (0.65 (7%) versus 0.39 (11%) L/min, respectievelijk, $p < 0.01$). Verder werd gevonden dat de gemiddelde orale opname duur (orale absorptie) van midazolam sneller was na een bariatrische operatie (23 (20%) versus 51 (15%) minuten, $p < 0.01$) en dat de orale biologische beschikbaarheid (F) onveranderd was (0.54 (9%)) in vergelijking met vóór de operatie. Het centrale en perifere verdelingsvolume van midazolam waren over het algemeen lager in patiënten na een bariatrische ingreep dan vóór de ingreep ($p < 0.05$).

Uit deze studie kan geconcludeerd worden dat patiënten na een bariatrische ingreep een 1,7 keer verhoogde midazolam klaring (CL) hebben in vergelijking met vóór de ingreep. Dit kan mogelijk verklaard worden door een toename van activiteit van het CYP3A enzym systeem en/of een verbetering van de lever doorbloeding. Verder kan worden geconcludeerd dat hoewel de orale opname duur sneller was, de biologische beschikbaarheid (F) van midazolam onveranderd was na een bariatrische ingreep. Deze laatste uitkomst in combinatie met een verhoogde klaring (CL) wijst mogelijk op een verhoging van de fractie van de midazolam dosis die ontsnapt aan het CYP3A metabolisme in de darmwand (F_G).

Op basis van de uitkomsten van Hoofdstuk 6 wordt verondersteld dat de fractie van de midazolam dosis die ontsnapt aan het CYP3A metabolisme in de darmwand (F_G) verhoogd is in patiënten na een bariatrische operatie in vergelijking met morbide obese patiënten vóór een dergelijke operatie. Kennis omtrent de precieze invloed van een bariatrische ingreep op de CYP3A activiteit in de lever en darmwand (en dus op de fractie van een geneesmiddel dat ontsnapt aan het lever (F_H) en darmwand metabolisme (F_G), respectievelijk) kan van grote waarde zijn voor andere geneesmiddelen, aangezien ongeveer 30% van alle geneesmiddelen via het CYP3A enzym systeem worden gemetaboliseerd²². Om deze reden hadden we in **Hoofdstuk 7** het doel om de farmacokinetiek van zowel midazolam als zijn CYP3A metaboliet, 1-OH-midazolam, na orale en intraveneuze toediening te beschrijven in morbide obese patiënten vóór en na een bariatrische operatie²³.

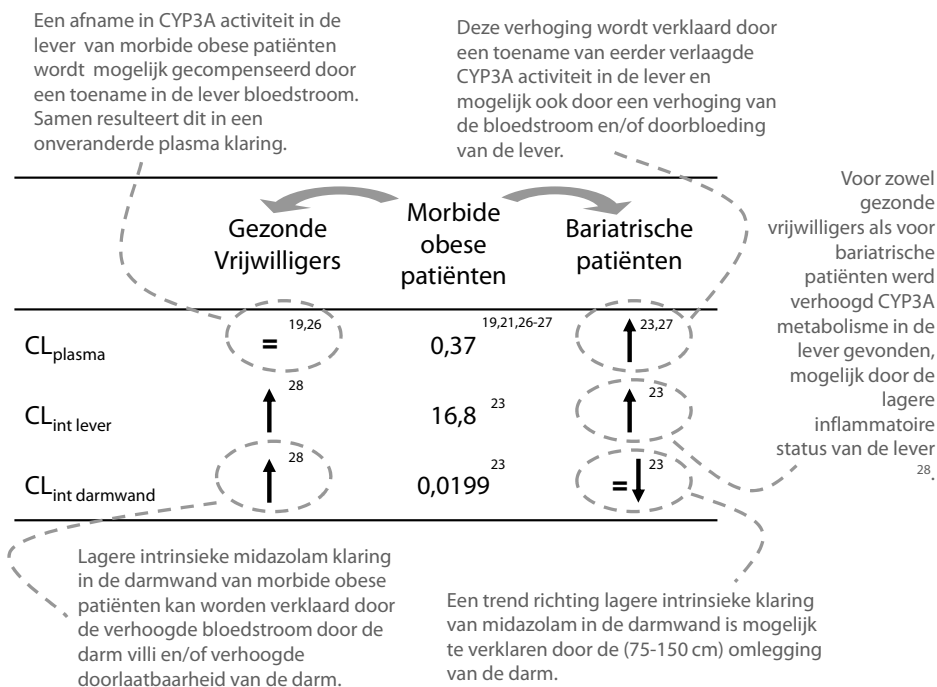
Voor deze studie werd een semi-fysiologisch gebaseerd farmacokinetiek (semi-PBPK) model toegepast. In dit model werd met het CYP3A omzetting van midazolam naar 1-OH-midazolam in zowel de darmwand als in de lever rekening gehouden. Met het semi-PBPK model werd gevonden dat intrinsieke leverklaring ($CL_{\text{int lever}}$) in patiënten na een bariatrische ingreep 1,5 keer hoger is in vergelijking met morbide obese patiënten vóór de operatie ($p < 0.01$). Dit leidt tot een lagere F_H van midazolam voor bariatrische

patiënten. Daarentegen liet de intrinsieke midazolam klaring in de darmwand ($CL_{int \text{ darmwand}}$) een trend van lagere waarden zien in bariatrische patiënten in vergelijking met morbide obese patiënten. Hoewel vermeld moet worden dat de $CL_{int \text{ darmwand}}$ voor beide groepen erg laag was. Deze lage $CL_{int \text{ darmwand}}$ waarden resulteerden in F_G waarden van bijna 1 voor beide patiënten groepen, terwijl met name de morbide obese patiënten grote inter-individuele variabiliteit lieten zien.

De resultaten van dit model zijn vervolgens gebruikt om inzicht te krijgen in hoeverre deze uitkomsten ook van toepassing zijn op andere CYP3A substraat geneesmiddelen met behulp van simulaties met het software pakket SimCYP²⁴. In de morbide obese patiënten populatie van het software pakket SimCYP werd de CYP3A hoeveelheid in de lever 1,5 vergroot, om de gevonden 1,5 keer verhoogde intrinsieke midazolam leverklaring na te bootsen. Voor de CYP3A substraten ciclosporine, alprazolam en triazolam (alle drie met een lage lever extractie ratio) resulteerde dit in een verhoging van de plasmaklaring tussen de 1,30-1,41. Voor midazolam (met een middelmatig lever extractie ratio geneesmiddel) resulteerde deze verhoging in slechts een 1,22 keer toename van de plasmaklaring. Deze factor van 1,22 is lager dan de 1,7 keer verhoogde plasma klaring welke we vonden in Hoofdstuk 6. Dit kan verklaard worden door het feit dat de klaring van midazolam niet alleen door de activiteit van het CYP3A enzym systeem in de lever wordt bepaald, maar ook door een ander proces (bijvoorbeeld de mate van bloedstroom door de lever).

De resultaten van Hoofdstuk 7 in combinatie met de uitkomsten van Hoofdstuk 5 en 6 zijn samengevat in Figuur 1. In dit figuur worden de midazolam plasma klaring (CL_{plasma}) en de intrinsieke bloedklaringen van de lever ($CL_{int \text{ lever}}$) en de darmwand ($CL_{int \text{ darmwand}}$) van morbide obese patiënten (middelste kolom) vergeleken met die van gezonde vrijwilligers (linker kolom) en patiënten één jaar na een bariatrische operatie (rechter kolom). Het figuur laat zien dat de verhoging van de midazolam plasma klaring (CL_{plasma}) na een bariatrische operatie niet alleen verklaard wordt door een normalisatie van de eerder verlaagde CYP3A activiteit in de lever (vanwege morbide obesitas), maar dat er ook een niet-CYP3A gerelateerd proces bijdraagt aan deze verhoging in de midazolam plasma klaring (Hoofdstuk 6)²⁵. Dit andere proces zou een verhoogde bloedstroom door de lever (Q_H) of verhoogde doorbloeding van de lever kunnen zijn. Een verhoogde lever bloedstroom in morbide obese patiënten in vergelijking met gezonde vrijwilligers kan mogelijk ook de gelijke midazolam plasma klaring in deze beide groepen verklaren (Hoofdstuk 5 en Figuur 1)¹⁹.

Op basis van de studie in Hoofdstuk 7 kan worden geconcludeerd dat een semi-PBPK model midazolam en 1-OH-midazolam concentraties over de tijd adequaat kan beschrijven na zowel orale als intraveneuze dosis. Met behulp van dit model werd aangetoond dat in bariatrische patiënten de intrinsieke metabole CYP3A capaciteit van de lever hoger is dan in morbide obese patiënten vóór de operatie. De CYP3A capaciteit van de darmwand, daarentegen, liet in bariatrische patiënten een trend van lagere waarden zien, mogelijk door de 75-150 cm omlegging van het eerste deel van de darm.



Figuur 1 Een overzicht van de resultaten van midazolam plasmaklaring (CL_{plasma} (L/min)), intrinsieke bloedklaring in de darmwand ($CL_{\text{int darmwand}}$ (L/min)) en intrinsieke bloedklaring in de lever ($CL_{\text{int lever}}$ (L/min)) in morbid obese patiënten in vergelijking met (niet-obese) gezonde vrijwilligers en bariatrische patiënten. Dit zijn de resultaten uit Hoofdstuk 5, 6 en 7²⁶⁻²⁸. De grijze pijlen geven de richting van vergelijking aan.

PERSPECTIEVEN EN CONCLUSIES

In dit proefschrift zijn twee geneesmiddelen bestudeerd in morbid obese patiënten en patiënten na een bariatrische operatie door middel van de uitvoer van adequate klinische studies. Deze studies hebben geleid tot wetenschappelijk onderbouwde doseeradviezen voor cefazoline en midazolam in deze patiëntengroepen (zie Appendix I).

Het zal zeer tijdrovend en kostbaar zijn om op deze wijze voor elk geneesmiddel afzonderlijk wetenschappelijk onderbouwde doseeradviezen te ontwikkelen. Daarom is het een interessante vraag hoe we dit proces kunnen versnellen. Een methode is om te inventariseren of de farmacokinetiek modellen die we per geneesmiddel hebben ontwikkeld, informatie bevatten die specifiek is voor de onderzochte populatie, zogenaamde systeem specifieke informatie (bijvoorbeeld een veranderde CYP3A activiteit in de lever in patiënten met toenemende obesitas). Dergelijk systeem specifieke informatie kan vervolgens gebruikt worden voor het voorspellen van de farmacokinetiek voor andere (nog niet onderzochte) geneesmiddelen (die bijvoorbeeld ook via het CYP3A enzym systeem worden afgebroken). Dit concept werd reeds eerder onderzocht en toegepast

voor UGT2B7 glucuronidering en renale (nier) klaring in kinderen²⁹⁻³⁵. In Hoofdstuk 2 hebben we voor obese patiënten geprobeerd eenzelfde aanpak toe te passen met behulp van een literatuuroverzicht. Dit overzicht wijst inderdaad op coherente trends qua invloed van obesitas per metabole of eliminatie route.

In dit proefschrift vonden we geen invloed van lichaamsgewicht op de klaring van cefazoline (Hoofdstuk 4), wat voornamelijk via glomerulaire filtratie en actieve tubulaire excretie in de nieren wordt uitgescheiden^{15, 18}. Deze bevindingen komen overeen met de algemene trend voor glomerulaire filtratie uit het literatuuroverzicht van Hoofdstuk 2, waarin glomerulaire filtratie een kleine verhoging of geen verandering laat zien onder invloed van obesitas.

Wat betreft midazolam suggereert het overzicht van Hoofdstuk 2 dat het geneesmiddelmetabolisme via CYP3A in de lever is verlaagd in patiënten met obesitas. Dit is met name gebaseerd op de orale klaring waarden (CL/F) van CYP3A geneesmiddelen gevonden uit eerdere studies in obese versus niet-obese patiënten. In de studie van Hoofdstuk 5 met het CYP3A substraat midazolam, vonden we geen verschil in midazolam klaring (CL) tussen morbide obese patiënten en gezonde vrijwilligers, maar wel een hogere biologische beschikbaarheid (F) in morbide obese patiënten (60 versus 28% respectievelijk). Dit resulteert dus inderdaad in een sterk afgenomen orale klaring (CL/F) van midazolam in morbide obese patiënten, zoals gesuggereerd in het overzicht van Hoofdstuk 2. Onze resultaten komen overeen met eerder studies en dus kunnen we stellen dat uitkomsten voor één geneesmiddel voorspellend lijken te zijn voor andere geneesmiddelen die dezelfde klaring route hebben (zie ook Hoofdstuk 7).

In tegenstelling tot de resultaten voor klaring (CL), werd er een sterke invloed van lichaamsgewicht gevonden op het verdelingsvolume (V) van cefazoline en midazolam (Hoofdstuk 4 en 5) in morbide obese patiënten. Om de invloed van obesitas op het verdelingsvolume van een geneesmiddel beter op voorhand te kunnen voorspellen, lijken fysiologische gebaseerde farmacokinetiek modellen van grote waarde te kunnen zijn. In fysiologische gebaseerde farmacokinetiek (PBPK) modellen wordt rekening gehouden met de eigenschappen van een geneesmiddel (o.a. log D, eiwitbinding, weefsel partitie coëfficiënten, etc.) en die van het systeem (bijvoorbeeld de bloedstroom door de organen en de organengroottes in het lichaam van een morbide obese patiënt). Op dit moment zijn er verschillende software pakketten beschikbaar met dergelijke PBPK modellen, onder andere SimCYP^{24, 36}, en de voorspellende waarde van deze modellen op het gebied van het verdelingsvolume in morbide obese patiënten dient onderzocht te worden. Dergelijk concepten en PBPK modellen kunnen ook worden toegepast voor patiënten die een bariatrische operatie hebben ondergaan. Hoewel er al enkele stappen in deze richting zijn gemaakt³⁷ is er nog een groot tekort aan kennis en informatie rondom deze patiënten. Daarom zal deze integrale aanpak in nauwe samenwerking moeten plaatsvinden met groepen die klinische studies in morbide obese patiënten en

bariatrische patiënten uitvoeren. Op die manier kunnen de PBPK modellen van meer informatie worden voorzien, waardoor het voorspellend vermogen van deze modellen wordt verbeterd.

In de toekomst blijft het uitvoeren van klinische studies naar geneesmiddelendoseringen in morbide obese patiënten en bariatrische patiënten dus essentieel. Op basis van onder andere onze ervaringen met de verschillende onderzoeken in dit proefschrift komen we tot een aantal adviezen voor toekomstige klinische studies in morbide obese en bariatrische patiënten.

Ten eerste is het belangrijk dat er naast geneesmiddel concentraties (farmacokinetiek) ook gegevens over klinische effecten (farmacodynamiek) worden verzameld. Ten tweede adviseren we de fysiologische eigenschappen in kaart te brengen van zowel obese als bariatrische patiënten (hartminuut volume, bloedstromen door organen, etc.), zodat PBPK modellen kunnen worden verbeterd. Ten derde adviseren we om, indien mogelijk, de farmacokinetiek en farmacodynamiek van een geneesmiddel na zowel orale als intraveneuze toediening te bestuderen. Daardoor kunnen de klaring (CL), het verdelingsvolume (V) en de biologische beschikbaarheid (F) van een geneesmiddel onafhankelijk van elkaar bepaald worden waardoor deze gegevens mogelijk ook voor andere geneesmiddelen kunnen worden gebruikt. Tenslotte adviseren we om in nieuwe studies in obese patiënten te streven naar een zo groot mogelijke variatie aan patiënten van overgewicht (BMI>25) tot en met super obesitas (BMI> 60) en, in het geval van bariatrische patiënten, een zo groot mogelijke variatie in tijd na operatie (>1 maand - >10 jaar) en type bariatrische operatie. Op deze manier zal de relatie tussen een covariaat, zoals lichaamsgewicht, 'mate van obesitas' of 'type bariatrische ingreep', op bijvoorbeeld het verdelingsvolume van een geneesmiddel beter in kaart kunnen worden gebracht. Op basis van deze concepten verwachten we sneller te komen tot wetenschappelijk onderbouwde doseeradviezen voor alle geneesmiddelen voor obese en bariatrische patiënten.

Kortom, de meest efficiënte weg naar wetenschappelijk onderbouwde doseeradviezen in morbide obese patiënten en patiënten na een bariatrische operatie is om goed opgezette klinische studies uit te voeren waarin wordt onderzocht of systeem specifieke eigenschappen van de populatie kunnen worden afgeleid. Vervolgens moet worden bestudeerd of deze systeem specifieke gegevens de invloed van morbide obesitas en een bariatrische operatie op andere geneesmiddelen kan voorspellen.

Om dit doel te bereiken dienen er klinische studies worden uitgevoerd met een wijd bereik aan patiënten: van overgewicht (BMI>25 kg/m²) tot en met super obesitas (BMI 100+ kg/m²) en, in het geval van bariatrische patiënten, verschillende typen bariatrische ingrepen en verschillende periodes na een ingreep. Verder is het belangrijk dat er binnen deze klinische onderzoeken geneesmiddelconcentraties over de tijd (farmacokine-

tiek), klinische effecten (farmacodynamiek) en fysiologische parameters (bijvoorbeeld bloedstromen door organen, eiwit binding, etc.) worden gemeten. Tenslotte dienen er PBPK(-PD) modellen ontwikkeld te worden welke in staat zijn om de farmacokinetiek en fysiologische gegevens, en mogelijk ook de farmacodynamische gegevens te integreren. Dergelijke geïntegreerde modellen zullen de voorspelling van de invloed van (morbide) obesitas en bariatrische operatie op nog niet bestudeerde geneesmiddelen verbeteren en versnellen. Met dit proefschrift hopen we een bijdrage te hebben geleverd aan dit relevante onderwerp.

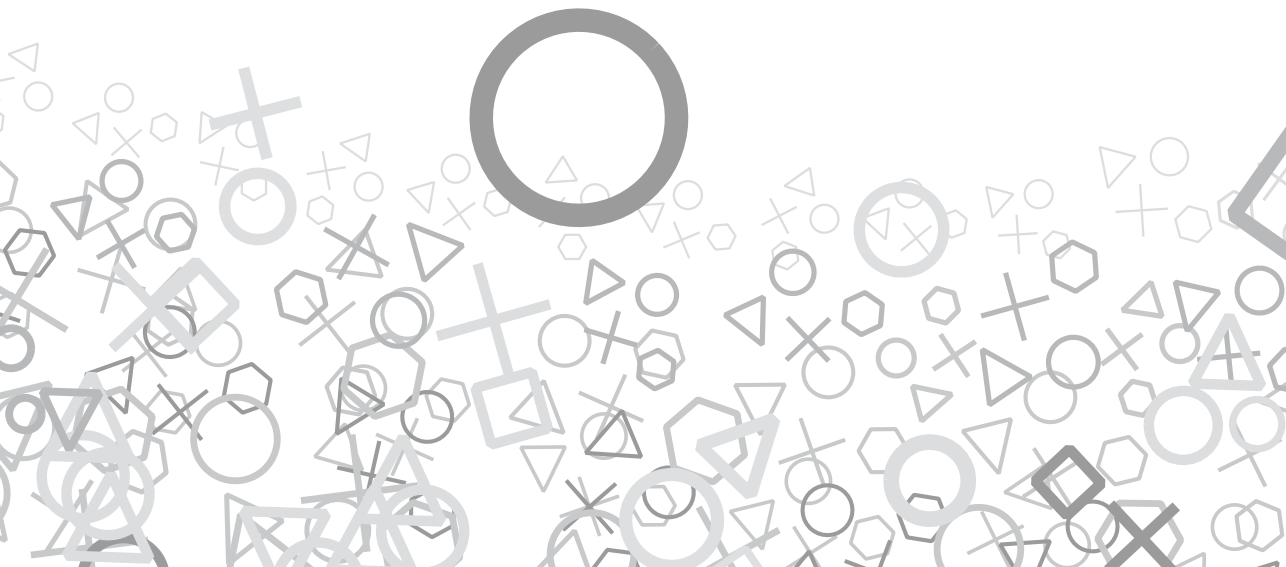
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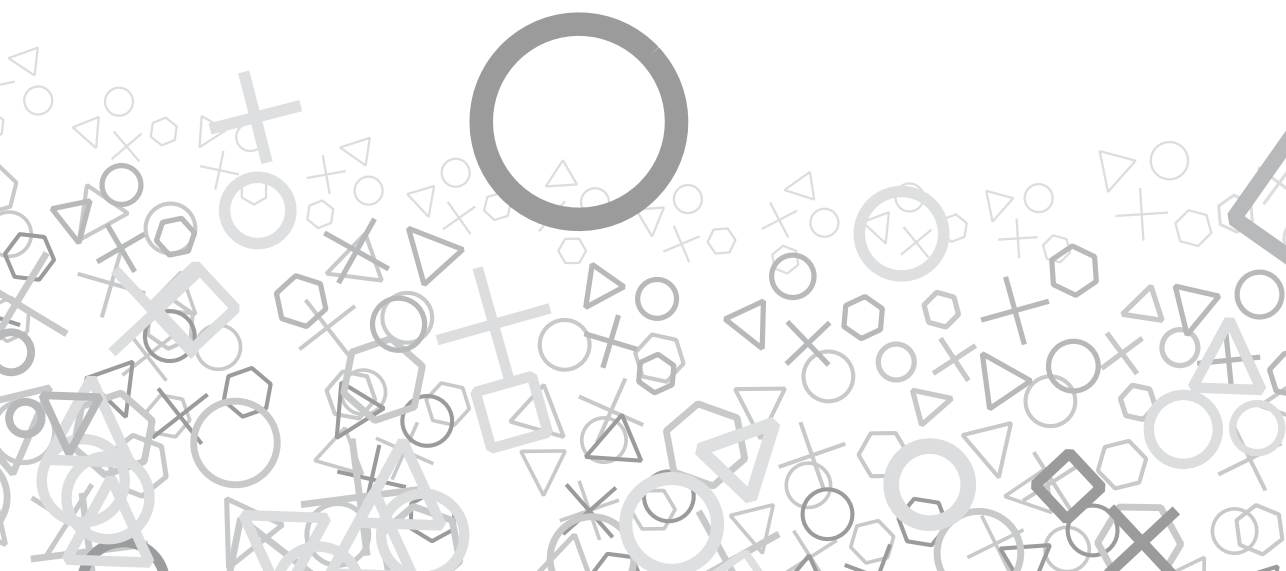
Appendices





APPENDIX I

Evidence-based dosing recommendations



Based on the results of the studies in this thesis together with other relevant studies currently available in the literature, evidence-based dosing guidelines for morbidly obese patients and in case of midazolam also for bariatric patients can be composed. A dosing advice monograph consists of a discussion of the relevant available literature (evidence) and a proposed dosing advice. Then, these monographs should be discussed by an expert panel consisting of clinical pharmacologists, hospital pharmacists and medical specialists (expert opinion). After finishing this process, the final dosing advice can be included in the Informatorium Medicamentorum of the knowledge center of the Royal Dutch Society for Pharmacy (KNMP), which can be accessed by Dutch pharmacists and physicians. For the drugs studied in this thesis, monographs were prepared and are presented below in a summarized version.

1.1 CEFAZOLIN IN MORBIDLY OBESE PATIENTS

Advice

For prophylaxis in morbidly obese patients ($\text{BMI} > 40 \text{ kg/m}^2$) in the Netherlands, 2 grams of cefazolin will lead to adequate subcutaneous ISF levels for a minimal inhibitory concentration (MIC) of 1 mg/L. Repeat this dose after 4 hours in case the surgical procedures lasts longer than 4 hours. This dosing advice is based on patients with a body weight range of 107 kg tot 175 kg ($\text{BMI} 40 \text{ kg/m}^2$ tot 57 kg/m^2)¹.

Discussion

This advice is based on Chapter 4 and three other studies on the pharmacokinetics and/or pharmacodynamics of cefazolin in (morbidly) obese patients¹⁻⁴. To evaluate the influence of morbid obesity on cefazolin target site concentrations in Chapter 4 unbound cefazolin concentrations at the interstitial space fluid (ISF) of the subcutaneous adipose tissue were measured using microdialysis. It was found that cefazolin subcutaneous tissue penetration is reduced in morbidly obese patients in comparison to non-obese patients. Using Monte Carlo simulations it was shown that unbound ISF cefazolin will remain above 1 mg/L until 120 minutes post dose in morbidly obese patients (see Figure 4 in Chapter 4). However, in case higher MIC values apply (2 or 4 mg/L), unbound ISF cefazolin concentrations will drop below the MIC values faster than in non-obese patients and redosing or increasing the initial cefazolin dose may be necessary¹.

While it has been suggested before that a dose of 1 gram cefazolin as prophylaxis is inadequate for the prevention of surgical site infections in morbidly obese patients⁴, Edmiston *et al.* measured active cefazolin concentrations in 3 patient groups (A= BMI 40-50; B= BMI 50-60 en C= $\text{BMI} > 60 \text{ kg/m}^2$) after a cefazolin 2 gram intravenous dose and an additional dose after 3 hours in tissue biopsies at closure of the surgical wound. They

reported that 48.1%, 28.6% en 10.2% of the active cefazolin concentrations of Group A, B and C, respectively, reached an MIC > 8 µg/mg ³. However, these results cannot be easily extrapolated to the Dutch (and European) situation, while in the Netherlands generally an MIC₉₀ of 1 mg/L is considered (MIC at which 90% of all *s. aureus* species is susceptible to cefazolin 1 mg/L). In addition, the American guideline advises a prophylactic cefazolin dose of 2 gram for patients >80 kg and 3 grams for patients >120 kg ⁵. This advice is mainly supported by studies applying MIC values of 4 and 8 mg/L and the consideration that cefazolin is inexpensive and well-tolerated. As mentioned before, due to the different MIC values applied, this advice may not be applicable for the Dutch (and European) situation.

In conclusion, based on these studies a 2 gram cefazolin prophylactic dose is sufficient for morbidly obese patients in the Netherlands in case of an MIC of 1 mg/L. As these studies predominantly involved morbidly obese patients, it remains unclear how cefazolin should be dosed in overweight and obese patients.

1.2 MIDAZOLAM IN OBESE AND MORBIDLY OBESE PATIENTS

Advice based on pharmacokinetic considerations only

First of all, midazolam half-life in (morbidly) obese patients strongly increases with body weight, which may cause midazolam effect to last longer. This is particularly important to consider for intravenous administration of midazolam.

- Oral dose administration: Same dose as in non-obese adults (Figure 4 of Chapter 5).
- Intravenous bolus dose: To reach the same maximum concentration as in non-obese health volunteers, for (morbidly) obese patients total body weight should be used (calculate dose based on mg/kg dose * total body weight) for a mg/kg dose that would be used in a non-obese patient, see Figure 1a
- Intravenous continuous infusion: For a morbidly obese patient the body weight of a non-obese individual (i.e. 75 kg) can be used for an mg/kg dose that would be used for non-obese patients. To reach steady state concentration with in approximately 1 hour, higher infusion rates should be applied, see Figure 1b.

Discussion

In addition to the study of Chapter 5, one other study evaluated the pharmacokinetics of midazolam in (morbidly) obese patients after both oral and intravenous administration ⁷. In both studies parameters estimates for the obese and non-obese study population, for midazolam central volume of distribution (mean of 44 L vs 21 L in Brill *et al.* and 58 L vs 38 L in Greenblatt *et al.*, respectively), total volume of distribution (322 L versus 78 L ⁶ and 311 L vs 114 L ⁷) and systemic clearance (0.36 vs 0.36 L/min ⁶ and 0.47 vs 0.53 L/

min⁻¹) were in good agreement. However, the estimates for oral bioavailability (F) differed between the studies. Brill *et al.* found an oral bioavailability of 60% versus 28% ($p < 0.001$) and Greenblatt *et al.* of 42% versus 40% ($p > 0.05$). This difference may be explained by the studied population, i.e. the mean body weight of the obese population of Greenblatt *et al.* was 117 ± 8 kg ($n=20$) in comparison to 144 ± 22 kg ($n=20$) for Brill *et al.*

Due to the substantial increase of volume of distribution with body weight, one should consider a strong increase in the midazolam half-life in (morbidly) obese patients which may cause midazolam effects to last longer than in non-obese patients. The increased half-life also significantly prolongs the time to reach the steady state concentration in (morbidly) obese patients. For this reason, for midazolam continuous infusion, initially higher infusion rates are advised based on dose simulations. For a 145 kg patient, higher infusion rates for a period of 8 hours are proposed to reach the steady state concentration with 1 hour (Figure 1). For non-obese healthy volunteers, a higher infusion rate for the duration of 2 hours is needed to reach steady state concentration within 1 hour.

Finally, it should be noted that the midazolam dosing advice is based on midazolam concentration-time data, while midazolam effects (sedation) have not been included in these studies. Although, no studies have looked into the concentration-effect relationship for midazolam in (morbidly) obese patients, a clear concentration effect relationship has been shown for the general adult and pediatric population⁸⁻⁹.

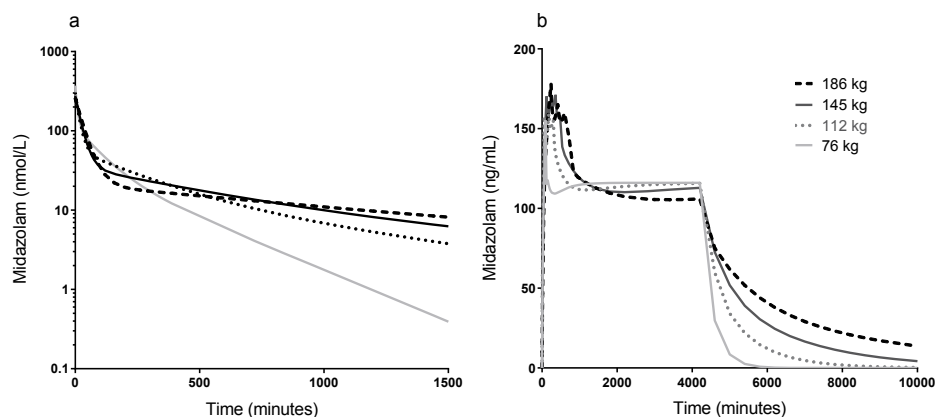


Figure 1 Simulated midazolam concentration time profiles for three morbidly obese patients of 112, 145 and 186 kg and one non-obese healthy volunteer of 76 kg after receiving:

(a) a 0.1 mg/kg total body weight (TBW) intravenous bolus dose (the concentration at $T=5$ minutes is 247 ng/ml for 76 kg; 236 ng/ml for 112 kg; 233 ng/ml for 145 kg; 229 ng/ml for 186 kg), (b) a continuous intravenous infusion of 2.5 mg/h (0.033 mg/kg/h*75kg) for the duration of 72 hours + a temporarily higher infusion rate to reach the steady state concentration within 1 hour (76 kg: 1h 7.5 mg/h, 1h 5 mg/h, 70h 2.5 mg/h; 112 kg: 1h 15 mg/h, 2h 7.5 mg/h, 2h 5 mg/h, 67h 2.5 mg/h; 145 kg: 2h 15 mg/h, 2h 10 mg/h, 2h 7.5 mg/h, 2h 5 mg/h, 64h 2.5 mg/h; 186 kg: 4h 15 mg/h, 3h 10 mg/h, 3h 7.5 mg/h, 2h 5 mg/h, 60h 2.5 mg/h). The simulations have been executed based on the population pharmacokinetic model by Brill *et al.*⁶

I.3 MIDAZOLAM IN GASTRIC BYPASS PATIENTS

Advice based on pharmacokinetic considerations only

- Oral dose administration: The same total dose in mg as in non-obese adults. Be aware of a decreased time to maximum concentration (T_{max}) and increased maximum concentration (C_{max}) in comparison to morbidly obese and matched control patients. In addition, midazolam effect will have shorter duration due to increased midazolam systemic clearance of which the clinical relevance is unclear (see Figure 3 of Chapter 6).
- Intravenous bolus dose: Dose based on mg/kg total body weight (TBW). Be aware of more rapid decrease in effect due to increased midazolam systemic clearance in gastric bypass patients, see Figure 2a.
- Intravenous continuous infusion: To reach a similar steady state concentration as in morbidly obese patients, a higher infusion rate should be applied since the level of the steady state concentration is determined by midazolam clearance (which is increased). In gastric bypass patients midazolam clearance was 1.68 times higher in comparison to morbidly obese and control patients (therefore, calculate the dose based on: mg/kg * 75 kg/hour * 1.68), see Figure 2b.

Discussion

Clinical studies into the PK of midazolam show that after a gastric bypass, oral absorption rate is increased, oral bioavailability is similar and systemic clearance substantially increases in comparison to morbidly obese and matched control patients¹⁰⁻¹². Based on these studies the above midazolam dosing advice is defined. There are several important factors which need to be considered when adjusting the midazolam dose for gastric bypass patients. Firstly, it is important to know exactly what type of bariatric surgery a patient underwent. There are several types of bariatric surgery (as described in the introduction of this thesis) of which each may have a different effect on the PK of midazolam (and thus a different consequence for dosing). The advice defined here is based on studies which predominantly included patients after a Roux- and Y gastric bypass (RYGB). In the study described in Chapter 6 also two gastric sleeve patients were included¹⁰, and therefore potentially, for this group the same midazolam dosing advice may be applied. Secondly, it should be noted that it remains unclear how the pharmacokinetics of midazolam changes over the course of time after a gastric bypass procedure. Possibly, there is a difference in midazolam PK in patients shortly after versus 10 year after a gastric bypass procedure, because some sort of adaption may occur. The studies included in this monograph investigated patients on average 1-1.9 year after a gastric bypass and no further studies are currently available. Finally, this midazolam dosing advice is based on two studies in which midazolam PK was compared with morbidly

obese patients before the surgery¹⁰ and matched control individuals¹¹. Until now, no studies have directly compared midazolam PK in gastric bypass patients with non-obese patients or healthy volunteers.

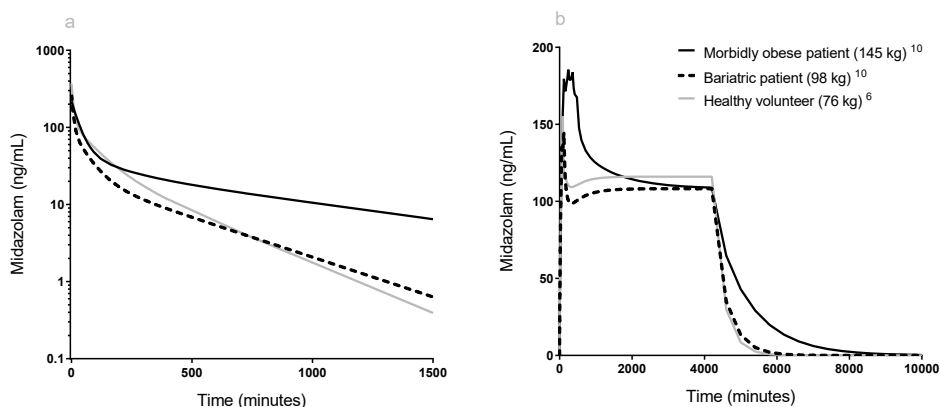


Figure 2 Simulated midazolam concentration-time profiles in a patient after a bariatric surgery, a 145 kg morbidly obese patients¹⁰ and a healthy volunteer (76 kg)⁶ after:

(a) an intravenous bolus dose of 0.1 mg/kg (7.5 mg for a healthy volunteer, 14.5 mg for a morbidly obese patients of 145 kg and 9.8 mg/kg for a bariatric patients of 98 kg). At T=5 minutes post dose midazolam concentrations are: 247 ng/ml, 194 ng/ml en 175 ng/ml, respectively. (b) a continuous intravenous infusion during 72 hours with temporarily higher infusion rates to quickly reach steady state concentration. (Bariatric patient: 1h 15 mg/h, 1h 10 mg/h, 70h 4.2 mg/h; healthy volunteer of 76 kg: 1h 7.5 mg/h, 1h 5 mg/h, 70h 2.5 mg/h; morbidly obese patient of 145 kg: 2h 15 mg/h, 2h 10 mg/h, 2h 7.5 mg/h, 2h 5 mg/h, 64h 2.5 mg/h). Simulations have been executed based on the population PK models from Brill *et al.*^{6,10}.

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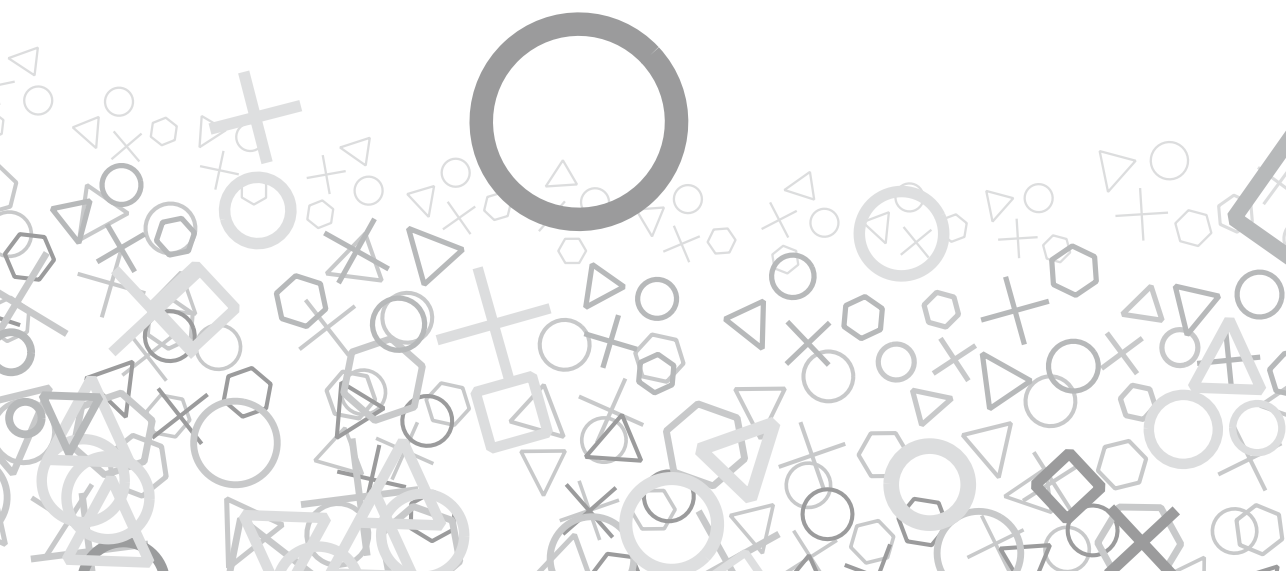


APPENDIX II

Curriculum vitae

List of publications

Nawoord/Acknowledgements



CURRICULUM VITAE

Margreke Brill was born on March 15, 1984 in Groningen, The Netherlands. After her graduation in 2002 from the Praedinius Gymnasium in Groningen, she lived in Paris for three months to study French and in Foundiougne, Senegal, for three months to perform volunteer work at a primary school. In 2003, she enrolled in Bio-Pharmaceutical Sciences at the Leiden University from which she graduated for her master's degree in the Pharmacology track in 2010. That year, she started as a PhD-student at the Clinical Pharmacy department of the St. Antonius Hospital, Nieuwegein, and the division of Pharmacology of the Leiden Academic Centre for Drug Research (LACDR) under supervision of Professor Catherijne A.J. Knibbe, resulting in this thesis.

Since September 2015, Margreke works as a post-doc at the Department of Pharmaceutical Biosciences of Uppsala University, Sweden, under supervision of Professor Mats Karlsson.

LIST OF PUBLICATIONS

van Rongen A, Kervezee L, Brill MJ, van Meir H, Den Hartigh J, Guchelaar HJ, Meijer JH, Burggraaf J, van Oosterhout F. Population pharmacokinetic model characterizing 24-hour variation in the pharmacokinetics of oral and intravenous midazolam in healthy volunteers. CPT:PSP 2015; Accepted for publication.

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Daarnaast ben ik uiteraard alle medewerkers en collega's van de afdelingen Klinische Farmacie, Heelkunde en Anesthesie zeer erkentelijk voor hun gastvrijheid, inhoudelijke bijdragen en praktische hulp tijdens mijn promotie onderzoek. In het bijzonder wil ik Mathieu Tjoeng, Vera Deneer, Rifka Peeters, Ewoudt van de Garde, Kees de Bruijn, Remco Harms, Sandra Otte en Petra Goené van de Klinische Farmacie noemen. Van de afdeling Heelkunde wil ik in het bijzonder Brigitte Bliemer, Silvia Samsom en Eric Hazebroek danken voor hun betrokkenheid bij het includeren van patiënten. Van de afdeling Anesthesie wil ik in het bijzonder de medewerkers van de PACU/Observatorium, onder leiding van Marion Brem, danken voor hun gastvrijheid en medewerking.

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