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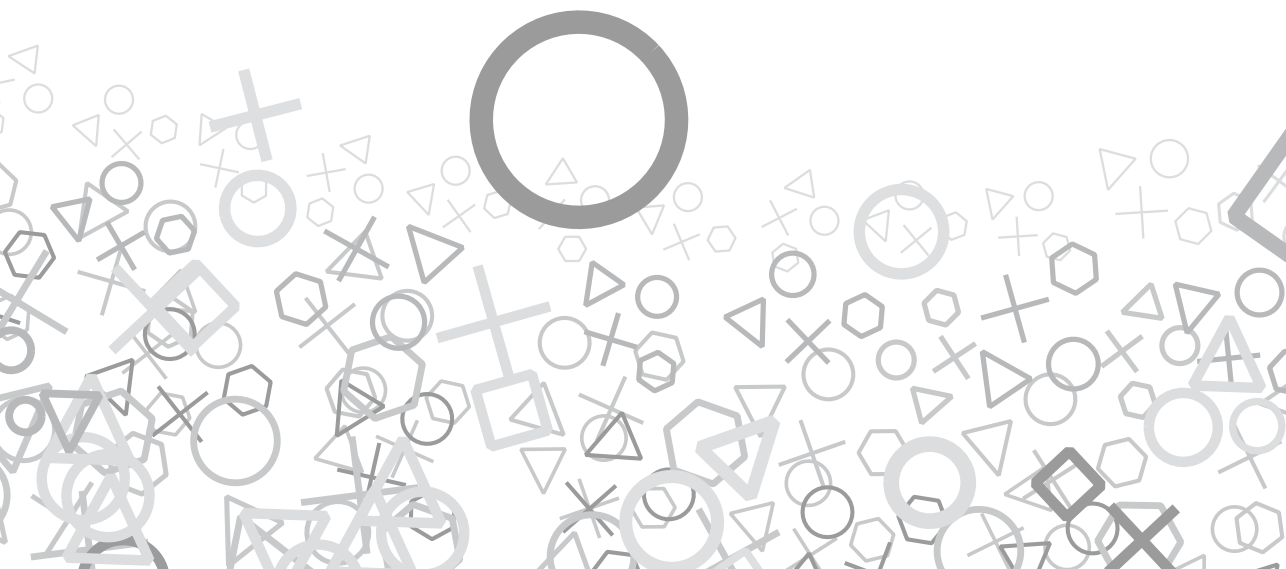
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CHAPTER 4

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 $47 \pm 6 \text{ kg/m}^2$) of which eight were evaluable, and seven non-obese patients (BMI of $28 \pm 3 \text{ kg/m}^2$) 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)).

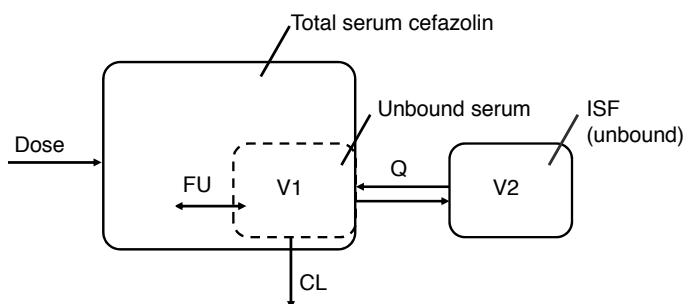


Figure 1 Schematic illustration of the population pharmacokinetic cefazolin model. The lines pointing towards a compartment indicate the type of observed data used for this compartment. CL= Clearance, FU= Fraction unbound, Q= Intercompartmental clearance, V1= Volume of distribution of central compartment,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

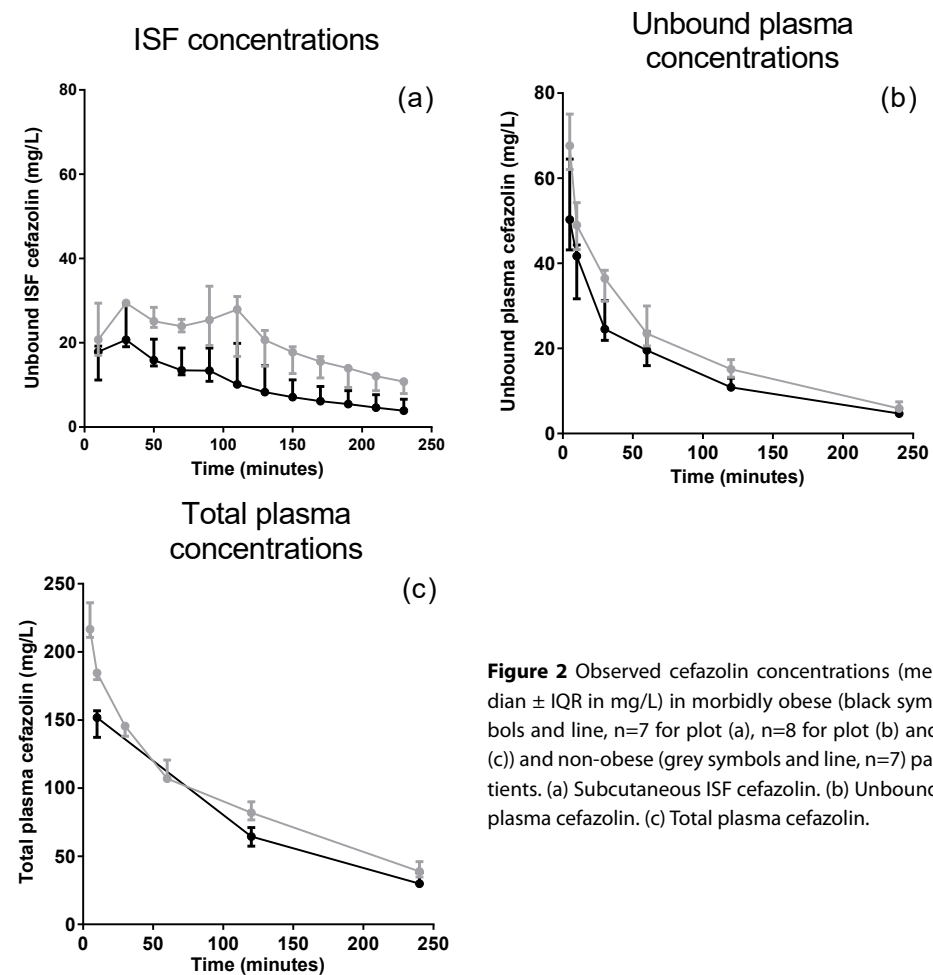


Figure 2 Observed cefazolin concentrations (median ± IQR in mg/L) in morbidly obese (black symbols and line, n=7 for plot (a), n=8 for plot (b) and (c)) and non-obese (grey symbols and line, n=7) patients. (a) Subcutaneous ISF cefazolin. (b) Unbound plasma cefazolin. (c) Total plasma cefazolin.

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 ce-fazolin amount in the central compartment, Kd= dissociation constant for ce-fazolin 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 ce-fazolin 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.

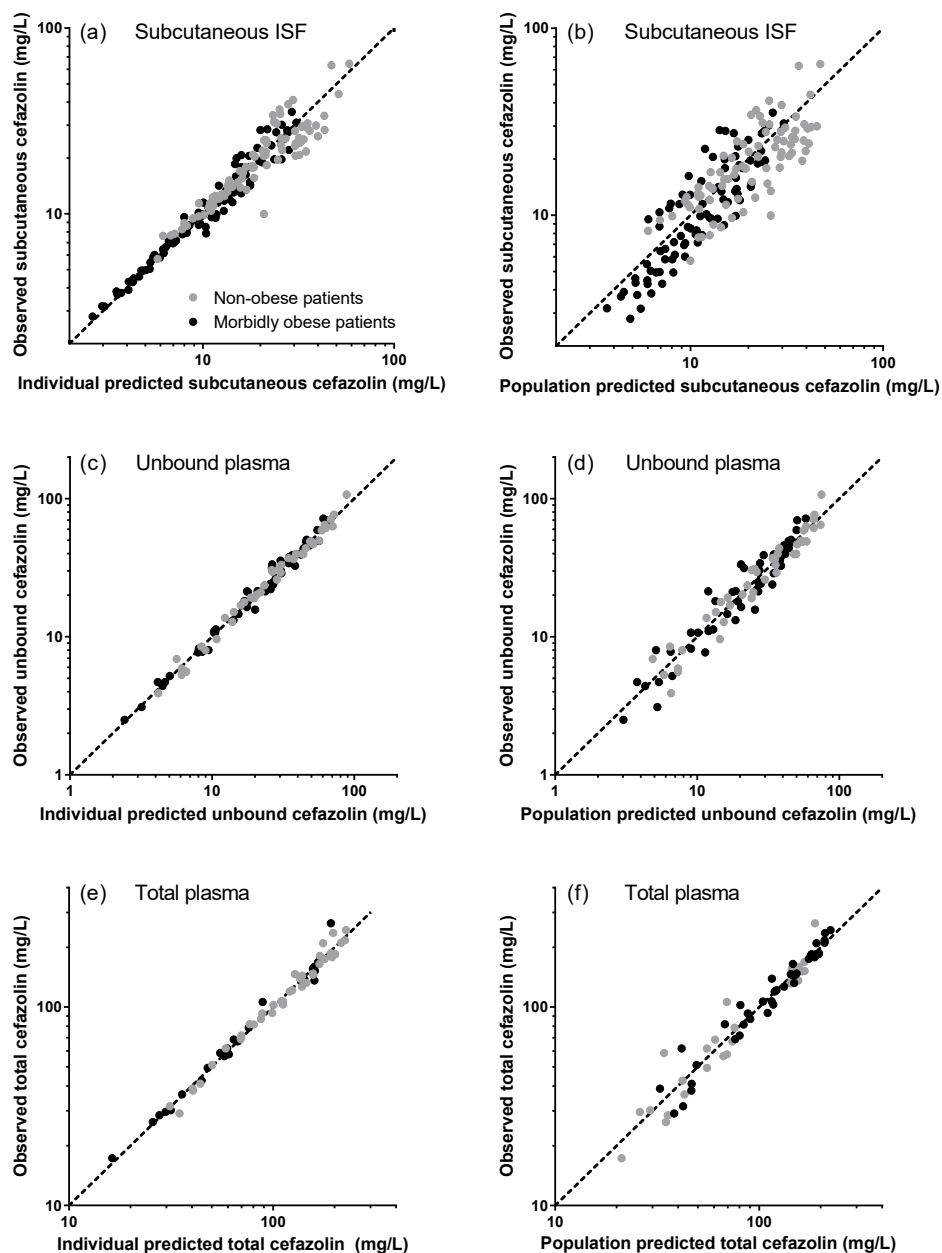


Figure 3 Observed versus individual predicted (a, c, e) and population predicted (b, d, f) cefazolin concentrations of subcutaneous ISF cefazolin (a and b), unbound plasma cefazolin (c and d) and total plasma cefazolin (e and f) in morbidly obese and non-obese patients. The dashed line represents the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

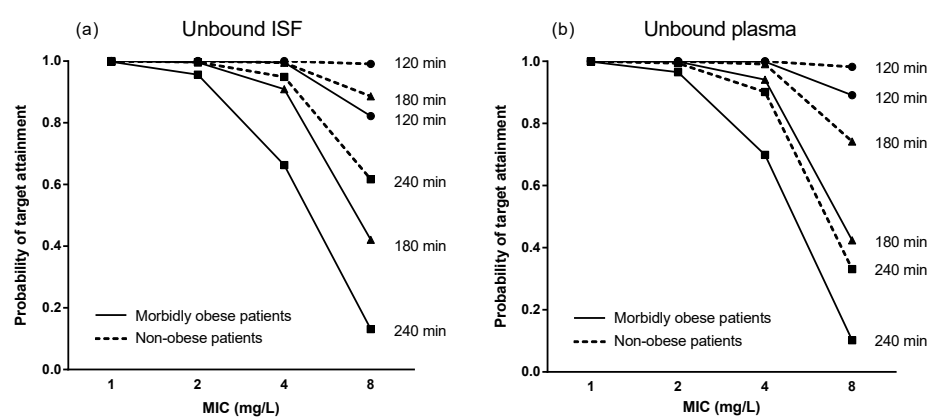


Figure 4 Probability of target attainment (PTA) at four different MIC values 120, 180 and 240 minutes after a 2 gram cefazolin iv dose in 5000 morbidly obese and 5000 non-obese Monte Carlo-simulated patients. (a) PTA of unbound cefazolin concentrations in the subcutaneous ISF. (b) PTA of unbound plasma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

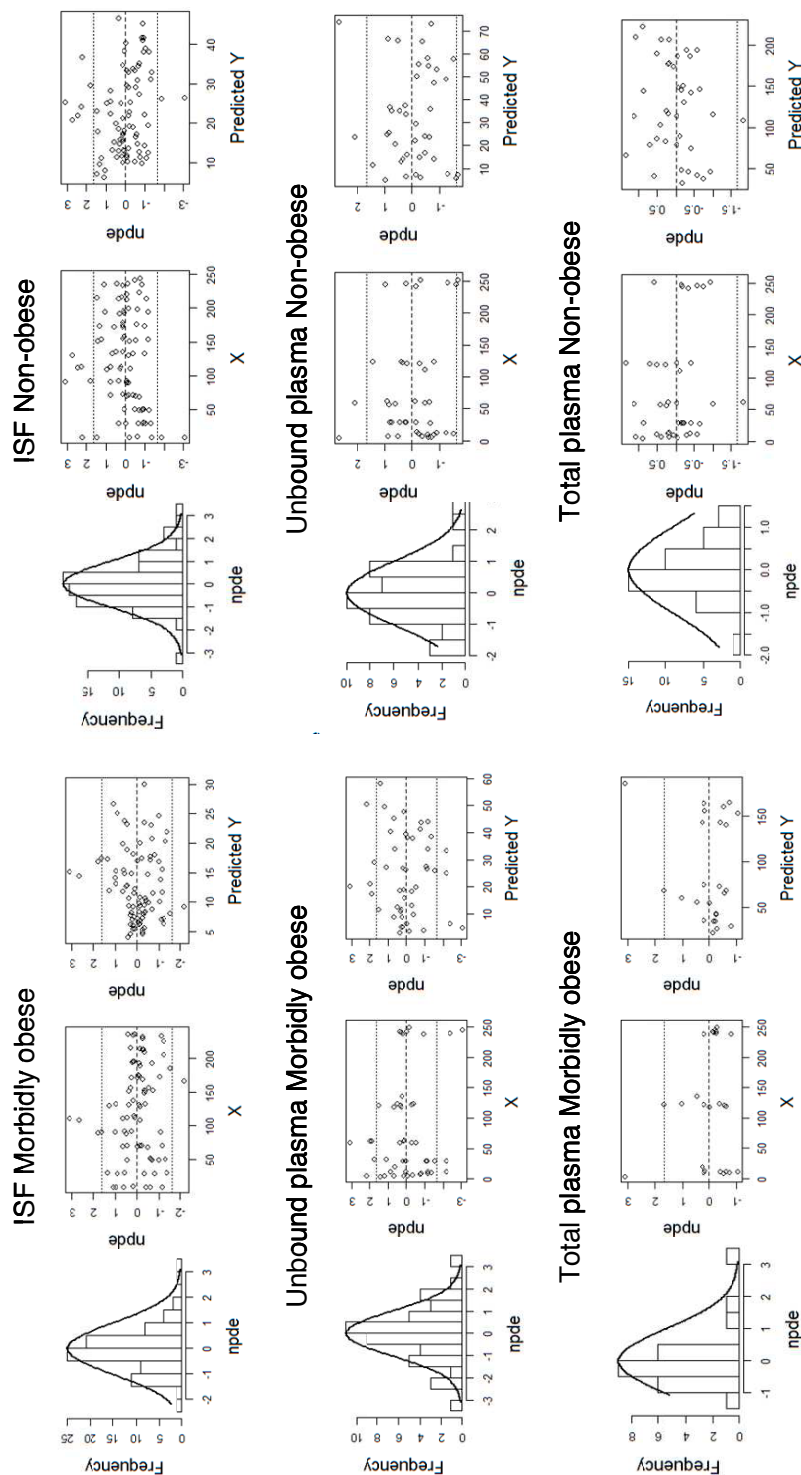


Figure S1 Results of the NPDE analysis. The first graph of each set of three graphs shows the histograms with the distribution of the NPDEs for cefazolin. The solid line depicts a normal distribution and the values below specify the mean and standard deviation of the observed NPDE distribution in the 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.

