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Research Article

Population Pharmacokinetic Modeling in the Presence of Missing Time-Dependent Covariates: Impact of Body Weight on Pharmacokinetics of Paracetamol in Neonates

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Abstract. Body weight is the primary covariate in pharmacokinetics of many drugs and dramatically changes during the first weeks of life of neonates. The objective of this study is to determine if missing body weights in preterm and term neonates affect estimates of model parameters and which methods can be used to improve performance of a population pharmacokinetic model of paracetamol. Data for our analysis were obtained from previously published studies on the pharmacokinetics of intravenous paracetamol in neonates. We adopted a population model of body weight change in neonates to implement three previously introduced methods of handling missing covariates based on data imputation, likelihood function modification, and full random effects modeling. All models were implemented in NONMEM 7.4, and population parameters were estimated using the FOCE method. Our major finding was that missing body weights minimally affect population estimates of pharmacokinetic parameters but do affect the covariate relationship parameters, particularly the one describing dependence of clearance on body weight. None of the tested methods changed estimates of between-subject variability nor impacted the predictive performance of the model. Our analysis shows that a modeling approach towards handling missing covariates allows borrowing information gathered in various studies as long as they target the same population. This approach is particularly useful for handling time-dependent missing covariates.

KEY WORDS: full random effects model; missing covariates; paracetamol; pediatric population.

INTRODUCTION

Time-dependent covariates are common in population pharmacokinetic (PK) and pharmacodynamic (PD) analysis (1). Conditional on the duration of PKPD studies, underlying mechanisms of time variance might include dynamic processes such as growth, aging, circadian rhythm, pregnancy, disease progression, and a changing environment. Age progression in pediatric populations affects anatomical, physiological, and biological processes over time (2,3). Renal clearance of antibiotics changes during the first weeks of life (4). Changes that take place in drug metabolism enzymes during fetal and postnatal development impact drug disposition (5). Variables of the comprehensive metabolic panel (e.g., albumin, creatinine, glucose, bilirubin) are often used as covariates accounting for functioning of body organs. As an example, in healthy term and late preterm newborns, peak plasma bilirubin levels often occur after 3 days of life (6). This peak is attributed to an increase in bilirubin production, in combination with covariate-driven maturation of the glucuronidation elimination capacity in the liver (7).

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Body weight is a physiological covariate correlated with many PKPD parameters. While relatively stable in adult subjects, it dynamically changes in the pediatric population. Newborns lose body fluid and fat during the first days of life which results in decreases in their body weight. Once food intake outweighs the initial loss of fluid and fat, body weight starts increasing (8). As much as 10% change in body weight can be observed within the first 3 days after birth in term newborn infants (9). A population model that characterizes physiological weight loss and weight gain during the first week of life in breastfed healthy term neonates was developed (10). Covariates that have effect on weight changes were identified: sex, gestational age, and mother's age. The model was able to accurately forecast individual weight changes up to 1 week. This model has been expanded recently to account for dose-dependent effects of supplemental feeding in late preterm and term neonates (11).

Paracetamol (N-acetyl-para-aminophenol, acetaminophen or APAP) is commonly administered in neonates and infants for the treatment of mild to moderate pain. Intravenous formulations only became available recently and are applied when enteral delivery is not possible, such as for postoperative pain relief (12). Results of studies on pharmacokinetics of intravenous APAP in neonates have been reported (13), and a population pharmacokinetic model for intravenous APAP in preterm and term neonates is available (14). Body weight was the principal predictor of intravenous APAP clearance and volume of distribution. The body weight was measured prior to APAP administration (current body weight), and blood samples were collected over 72 h. Given that the body weight dynamically changes in neonates and no body weight measurements were collected at times of APAP observed concentrations, the body weight was a time-dependent missing covariate.

Missing covariates are a common problem in PKPD data analysis. Deletion of data records with missing covariates may lead to biased results and violate the intent-to-treat principle (15). Many statistical approaches of handling missing data have been developed. Two major techniques have been adopted in nonlinear mixed effects modeling: imputation and likelihood function modification (16). Imputation substitutes the made-up data into the missing data and considers the imputed data as observed ones. Single imputation methods use mean or median of the distribution of observed covariates for substitution. More advanced single imputation methods apply regression on the observed covariates to infer the missing one. Statistically preferable are multiple imputation methods where the missing covariates are imputed multiple times in a random fashion to account for covariate variability (17). Assessment of performance of the multiple imputation methods in population PK analysis has been reported elsewhere (18). The likelihood method of handling missing covariates considers them as unobserved data and modifies the likelihood function (19,20). Missing time-dependent covariates create an additional challenge for existing methods of handling missing data as the covariate distribution changes with time. Most of these techniques become invalid as they assume stationary covariates. Only a few techniques of handling missing time-dependent covariates exist, and all of them are based on data imputation (21).

Modeling of covariate relationships has been expanded on fixed effects approaches where parameter-covariate equations are added into the model and parameter estimates are used for inferences about significant covariates (22). The full fixed effect model approach has been further generalized to the full random effects model (FREM) approach. In the FREM, covariates enter the model as observed variables and their distributions are modeled as random effects (23). Although FREM has been mostly applied for covariate selection, the presence of a random effect model for covariate distributions can be utilized in handling missing covariates by both imputation and likelihood techniques. This modeling approach is particularly useful if covariates are time-dependent. Then, the difference between a dependent variable and a covariate lies only in their intended use and interpretation; statistically and numerically, they are equivalent.

In a PKPD system, PK variables (e.g., drug plasma concentration) can be considered as explanatory variables for the PD response. Consequently, they are time-dependent covariates. PD observations missing individual time courses of plasma concentration are a common problem encountered in PKPD analysis that can be viewed as an example of missing time-dependent covariates. In this context, existing methods of describing PD data using the sequential approach are analogs of imputation and likelihood techniques (24).

The objective of this work is to determine if accounting for missing body weights in preterm and term neonates affects estimates of model parameters and improves performance of a population pharmacokinetic model of APAP administered intravenously. We applied previously published population models to describe APAP PK and time courses of body weights in neonates. Three techniques of handling missing covariates were adopted: single imputation, likelihood, and FREM.

METHODS

Data

Data for our analysis were obtained from previously published, prospective, single-center, open-label studies on the pharmacokinetics of intravenous APAP in neonates (14). Our original population consisted of patients less than 28 days postnatal age with a clinical indication for intravenous APAP who were admitted to the intensive care units at the Children's National Health System (Washington, DC, USA) and the University Hospital Leuven (Leuven, Belgium). Each subject's body weight was measured at birth and the time of first APAP dose only. The dynamics of body weight change in newborns were described by a model developed for data from healthy term newborns exclusively breastfed obtained retrospectively from a single-center study performed at the University Hospital of Basel (11). Since the model accounted for the body weight change during the first week of life for neonates of body weights no less 3270 g, the patients of postnatal age of 7 days and older and body weight at first dose less than 3.2 kg were excluded from our study population. If patient data lasted beyond 7 days after birth, they were truncated at day 7. The patient demographics and available covariate statistics are shown in

Table I. Major Covariates of Neonatal Population Included in the Analysis

Covariate	Median (range)
Number of neonates (*N*)	58
Birth weight (BW_0) (kg)	3.2 (2.5, 4.3)
Weight at first dose (CWT) (kg)	3.3 (2.5, 4.5)
Postnatal age at first dose (PNA_0) (days)	2.5 (1, 6)
Gestational age (GA) (weeks)	39 (34, 41)
Sex (SEX) (female/male)	23/35
Delivery mode (DELIV) (vaginal/cesarean)	42/16

Table I. Intravenous APAP was administered by 30-min infusions at 15 mg/kg/dose at 8-h intervals or 15-min infusions with a loading dose of 20 mg/kg, followed by maintenance doses at 6-h intervals. Each maintenance dose consisted of 5.0, 7.5, or 10 mg/kg. Blood samples were obtained from arterial lines at approximately 0, 1, 2, 4, 6, 8, 12, and 24 h after the first and last APAP doses or after each loading dose and just before maintenance doses. APAP plasma concentrations were determined using high-performance liquid chromatography with tandem mass spectrometry or UV detection, and the lower limit of quantification was 0.05–0.08 mg/L. Spaghetti plots of observed body weights and APAP plasma concentrations are shown in Fig. 1.

Population PK Model for APAP in Neonates

We adopted a population model describing APAP plasma concentrations in preterm and term neonates developed by Cook *et al.* (14). The fixed effects model consisted of one compartment with zero-order input $I(t)$ representing IV infusions of duration 30 min every 8 h.

$$\frac{dA}{dt} = I(t) - \frac{CL(t)}{V(t)} A \tag{1}$$

where $CL(t)$ is the APAP time varying clearance and $V(t)$ is the time varying volume of distribution. The time dependence of the PK parameters is a consequence of the time varying body weight $BW(t)$ that was identified as the only significant covariate for that patient population (14):

$$CL(t) = CL_0 \left(\frac{BW(t)}{\overline{BW_0}} \right)^{BW_{CL}} exp(\eta_{CL}) \tag{2}$$

and

$$V(t) = V_0 \left(\frac{BW(t)}{\overline{BW_0}} \right)^{BW_V} exp(\eta_V) \tag{3}$$

where CL_0, BW_{CL}, V_0, and BW_V are covariate relationship parameters, and η_{CL} and η_V are random variables accounting for between-subject variability of PK parameters that is not explained by the covariate. We assume that η_{CL} and η_V are independent and normally distributed with zero means and variances ω_{CL}^2 and ω_V^2, respectively. The centering parameter $\overline{BW_0} = 3.226$ kg equals the mean (in the log domain) of the neonate body weight at birth. Consequently, CL_0 and V_0 are typical values (in the log domain) of clearance and volume of distribution at birth. We also applied a proportional residual error model (15):

$$C_{ij} = \frac{A(t_{ij})}{V(t_{ij})} + \varepsilon_{Cij} \frac{A(t_{ij})}{V(t_{ij})} \tag{4}$$

where C_{ij} is the observed APAP plasma concentration for the i^{th} subject at time t_{ij} and ε_{Cij} are independent normal random variables with mean 0 and variance σ_C^2. In order to combine the PK model with a dynamic model for the body weight, we converted the time since the first dose (T) reported in the original data to the time since birth:

$$t = T + 24PNA_0 \tag{5}$$

where PNA_0 is the postnatal age at first APAP dose in units of days, and units for t and T are hours.

Population Dynamic Model for Body Weight in Neonates

To describe the time varying body weight in neonates, we adopted a population model introduced by Wilbaux *et al.* (10). According to this model, the change in the body weight in term neonates is determined by the time variant zero-order body weight gain rate $k_{in}(t)$ and first-order time variant body weight loss rate $k_{out}(t)$:

$$\frac{dBW(t)}{dt} = k_{in}(t) - k_{out}(t) BW(t) \tag{6}$$

with the initial condition set at time of birth $t = 0$ to

$$BW(0) = BW_0 \tag{7}$$

$$k_{in}(t) = \theta(t - T_{lag}) k_{inbase} exp(k_{inPNA} t) \tag{8}$$

and

$$k_{out}(t) = \frac{k_{outmax} t^{-H}}{T_{50}^{-H} + t^{-H}} + k_{outbase} exp(k_{outPNA} t) \tag{9}$$

where $\theta(t - T_{lag}) = 0$ if $t < T_{lag}$ and $\theta(t - T_{lag}) = 1$ if $t \geq T_{lag}$. The following covariate relationships were identified as having a significant impact on the body weight turnover parameters (10):

$$k_{inbase} = k_{inbase0}(1 + k_{inbaseGA}(GA - GA_0)) exp(\eta_{kinbase}) \tag{10}$$

where GA denotes gestational age in weeks, $GA_0 = 39.9$ weeks is the mean gestational age of the patient population from (10), $k_{inbase0}$ and $k_{inbaseGA}$ are covariate relationship

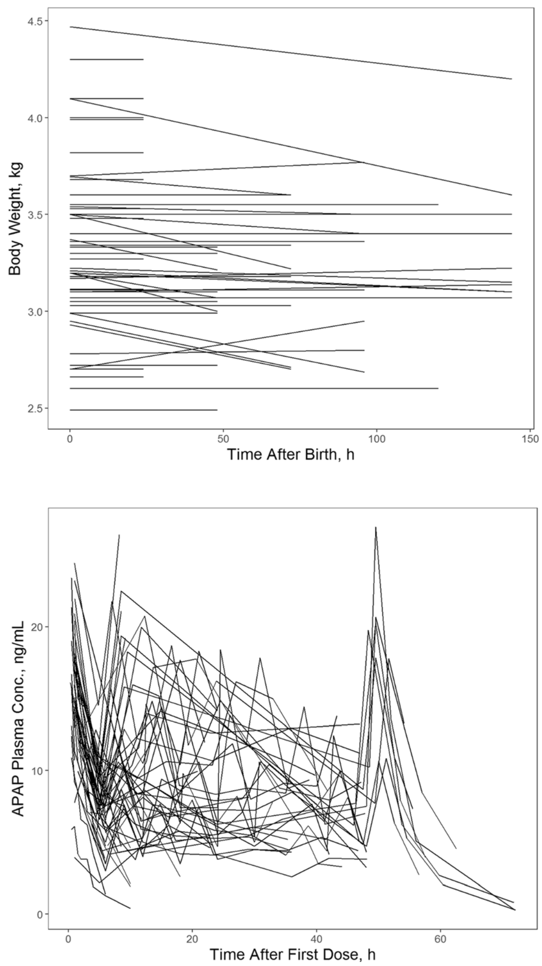


Fig. 1. Spaghetti plots of observed body weights (upper panel) and APAP plasma concentrations (lower panel)

parameters, and η_{kinbase} is a random variable, normally distributed, of mean zero and variance $\omega^2_{\text{kinbase}}$. In the original model, another covariate—mother's age—was identified as significant. In the absence of this information, the relationship in Eq. 10 holds true for a typical mother's age of 32 years.

$$BW_0 = W_0(1 + BW_{0SEX}(1 - SEX))(1 + BW_{0GA}(GA - GA_0))exp(\eta_{BW0}) \tag{11}$$

where SEX is a categorical covariate (0 for females and 1 for males), W_0, BW_{0SEX}, and BW_{0GA} are covariate relationship parameters, and η_{BW0} is a random variable normally distributed of mean zero and variance ω^2_{BW0}. As for k_{inbase}, Eq. 11 omits mother's age as a significant covariate.

$$T_{lag} = (1 - DELIV) T_{lagV} \exp\left(\eta_{TlagV}\right) + (DELIV) T_{lagC} exp\left(\eta_{TlagC}\right) \tag{12}$$

where $DELIV$ is a categorical covariate (0 for vaginal delivery and 1 for cesarean delivery), T_{lagV} and T_{lagC} are covariate relationship parameters, and η_{TlagV} and η_{TlagC} are independent random variables, normally distributed of mean zero and variances ω^2_{TlagV} and ω^2_{TlagC}, respectively. In agreement with Wilbaux *et al.* (10), a constant residual error model was selected for body weight measurements BW_{ij} at time t_{ij}:

$$BW_{ij} = BW\left(t_{ij}\right) + \varepsilon_{BWij} \tag{13}$$

where ε_{BWij} are independent normal random variables with mean 0 and variance σ^2_{BW}.

Imputation of Missing Body Weight

Since the neonate population described by the BW model consisted of healthy term newborns exclusively breastfed, whereas the population of neonates who received APAP consisted of term and preterm newborn patients admitted to the intensive care unit, we updated the estimates of the BW-related parameters W_0, BW_{0SEX}, BW_{0GA}, ω^2_{BW0}, and σ^2_{BW} by fitting the available BW data. The individual predicted BWs were imputed into missing BWs. We further call this technique IMPUT. This method is analogous to single imputation methods SI_{mode} and SI_{WT} discussed in (16) to account for a missing sex covariate. The APAP plasma concentrations with imputed BWs as complete covariate set were fitted by the PK model. The likelihood function of observing APAP plasma concentrations is provided in Appendix 1. NONMEM code for implementation of the imputation approach is provided in Supplementary Materials.

Likelihood Correction for Missing Body Weight

We adopted the concept of correcting the likelihood function to account for missing covariates introduced in (16) for missing sex information (the MOD method). The likelihood function for observed BWs was appended by the likelihood of missing observations assuming that the missing BWs are described by the population model developed by Wilbaux *et al.* (10). The likelihood function corrected for missing BWs was subsequently applied to fit the APAP plasma concentrations. We refer to this method as LIKELIHOOD. The likelihood function of observing APAP plasma concentrations is provided in Appendix 2. NONMEM code for implementation of LIKELIHOOD is provided in Supplementary Materials.

Full Random Effects Model

The full random effects model (FREM) was applied to account for the missing BWs. In essence, the observed APAP plasma concentrations $\boldsymbol{C} = \left\{C_{ij}\right\}_{1 \le i \le N, 1 \le j \le n_i}$ and observed BWs $\textbf{BW} = \left\{\text{BW}_{\text{ij}}\right\}_{1 \le \text{i} \le \text{N}, 1 \le \text{j} \le \text{m}_\text{i}}$ were fitted simultaneously by combined models Eqs. 1–4 and Eqs. 6–13. In this approach, the BWs in covariate relationships were described by the model-dependent variable $\text{BW}(t_{\text{ij}})$ rather than observed values BW_{ij}. This eliminated the need to account for missing BWs as all values were provided by the model. Statistically, both BWs and APAP concentrations were described by a joint model that considered both variables to be on par with each other. The only difference was that APAP plasma concentrations did not affect BWs, whereas

BWs influenced APAP pharmacokinetics. The likelihood function of observing APAP plasma concentrations and body weights is provided in Appendix 3. NONMEM code for implementation of the FREM approach is provided in Supplementary Materials.

Data Analysis

All models were implemented in NONMEM 7.4 (ICON Development Solutions, Ellicott City, MD). Population model parameters were estimated using the FOCE method with η-ε interaction. The goodness-of-fit plots and visual predictive check plots were obtained using R packages ggplot2, lattice, and vpc (27). For comparison of model performance, we used relative standard errors of parameter estimates, plots of observed vs. predicted values, and visual predictive checks.

RESULTS

Population PK Model for APAP with Current BW as Covariate

Our APAP data set differed from the one used by Cook *et al.* (14), but it was drawn from the same neonatal population. Therefore, the results of fitting the APAP plasma concentrations using the population model Eqs. 1–5 were expected to be similar to the published ones (14). The only departure from the published model was a different value of the centering parameter for covariate relationships Eqs. 2–3 (3.266 kg vs. 2.33 kg), as the former was calculated based on body weight at birth and the latter at time of first dose. The estimates of model parameters are shown in Table II. Both typical values and IIV parameters are similar. The differences between CL_0 and V_0 can be attributed to differences in centering in Eqs. 2–3. The observed vs. population predicted plot shown in Fig. S1 (Supplementary material) displays less bias in describing high APAP plasma concentrations than an analogous plot from (14). Observed vs. individual predicted plots are very similar and exhibiting the same degree of under prediction of the higher APAP plasma concentrations. The visual predictive check does not indicate any deviation of the distribution of model-predicted APAP plasma concentrations from the distribution of observed ones.

Population Model for BW

The set of observed BWs was restricted to birth and time of the first dose per subject. This sparse data prevented us from estimating all model parameters for our patient population of neonates. We selected only BW-related parameters W_0, $\mathrm{BW}_{0\mathrm{SEX}}$, $\mathrm{BW}_{0\mathrm{GA}}$, ω^2_{BW0}, and σ^2_{BW} for estimation, while the remaining model parameters were fixed at their values reported in Wilbaux *et al.* (10). The parameter estimates are shown in Table III. The estimates of W_0, $\mathrm{BW}_{0\mathrm{SEX}}$, and $\mathrm{BW}_{0\mathrm{GA}}$ were close to the analogous estimates from Wilbaux *et al.* (10). The expected higher variability in BW for our patient population was confirmed by a much higher estimate of ω^2_{BW0} than for the reported neonate population (0.0981 vs 0.01) as well as σ^2_{BW} (135 g vs. 32 g). Observed vs. predicted plots shown in Fig. S2 (Supplementary material) indicate that the model under-predicted higher body weights during the first day after birth, as demonstrated by the visual predictive check plot. This BW behavior is inconsistent with the time courses reported in Wilbaux *et al.* (10), where all subjects exhibited a decline in the first 2 days after birth. Consequently, the dynamic model for BW cannot account for the observed increase in BW during the first day. Figure 2 shows individual time courses for BW predicted by the model. They resemble the profiles shown in Wilbaux *et al.* (10), as most model parameters were fixed to the values form that report.

Comparison of Model Performances

The population PK model for APAP with current body weight as covariate served as a reference for comparisons. Three techniques were applied to account for missing BWs: imputation of missing values (IMPUT), correction of the likelihood objective function (LIKELIHOOD), and a full random effects model (FREM). The parameter estimates for these methods are presented in Table IV. Figure 3 shows estimates of fixed effect parameters CL_0, V_0, $\mathrm{BW}_{\mathrm{CL}}$, BW_{V}, and random effect parameters $\omega^2_{\mathrm{CL}}, \omega^2_{\mathrm{V}}$, and σ^2_{C} grouped by parameter. All techniques resulted in similar estimates for three out of four of the fixed effect parameters: CL_0, V_0, and BW_V were close to the reference estimates from Table II. The biggest differences were observed for estimates of $\mathrm{BW}_{\mathrm{CL}}$ that ranged between 1.67 and 2.74 with a reference value of 1.67. The estimates of the IIV variances ω^2_{CL} and ω^2_V were almost identical to the reference values with the exception of LIKELIHOOD, where ω^2_{CL} was equal to 47% of the reference (0.0439 vs. 0.0925). However, this estimate was imprecise with a %RSE of 80% that was much higher than the %RSE of the other methods, which did not exceed 20%. Visual inspection of observed vs. predicted plots shown in Figs. S3–S5 (Supplementary material) shows only small differences between all techniques. Population predictions for LIKELIHOOD and FREM are higher than observed APAP concentrations in their top range, a trend not observed for IMPUT and reference models. Individual predictions for all methods match reference ones. A similar observation applies to the visual predictive checks which showed only minute differences from the reference.

In contrast to IMPUT and LIKELIHOOD, FREM allowed for the simultaneous fit of APAP and BW data. The FREM estimates of BW-related fixed effect parameters W_0, BW_{0SEX}, and BW_{0GA} were almost identical to the reference estimates from Table III. The estimate of the IIV variance parameter ω^2_{BW0} was much greater than the reference (0.0858 vs. 0.00962). Observed vs. predicted plots shown in Fig. S6 (Supplementary material) are very similar to the reference for population predictions. However, individual predicted BWs are less correlated with observed ones than for the reference ($r^2 = 0.88$ vs. $r^2 = 0.95$). This is also reflected in the higher residual variability $\sigma_{\mathrm{BW}}{}^2$ in FREM vs. reference (0.0326 vs 0.0181).

DISCUSSION

The objective of this study was to assess the impact of time-dependent BW on APAP PK in neonates where the BW measurements were taken only once during the treatment. Since the key PK parameters volume of distribution and clearance depend on BW, one might expect that the estimates

Table II. The Estimates of the Reference Population PK Model Parameters and Their Standard Errors

Parameter	Estimate	SE (%RSE)
CL_0 (L/h)	0.556	0.0244 (4.3)
V_0 (L)	3.51	0.109 (3.1)
BW_{CL}	1.67	0.431 (25.8)
BW_V	1.11	0.266 (24.0)
ω_{CL}^2	0.0925 (30.4%)	0.0168 (18.2)
ω_V^2	0.0315 (17.7%)	0.00654 (20.8)
σ_C^2	0.0277 (16.6%)	0.00369 (13.3)

of their typical values and IIV will differ if the missing BW throughout the treatment will be accounted for. We applied a modeling approach where an existing model describing time courses of BW in neonates was used to infer missing observations. Three different techniques introduced previously to handle missing data were tested. Our major finding was that missed BWs minimally affect population estimates of PK parameters but do affect the covariate relationship parameters, particularly the one describing the dependence of clearance on BW. This relationship is of importance for model-based dose selection for neonates. Neither IMPUT nor FREM methods inflated nor reduced estimates of between-subject variability. LIKELIHOOD method reduced between-subject variability for clearance. We concluded that if the covariate relationships account for one observed BW at any time after birth and are kept constant, then the predictive performance of such model is as good as one for a model that accounts for BW at each observation time. The underlying reason is that although individual patient volume and clearance vary in time due to the BW-time dependence, the change of individual subject APAP plasma concentrations is of the same magnitude. Figure 4 shows a predicted BW time course of a subject who received the first APAP dose 3 days after birth. The BW increased 8% over the course of treatment, clearance 16%, and volume of distribution 8%. The difference in APAP plasma concentration from the reference was increasing in time to reach 36% after 72 h. Given that the %CV of the residual error for APAP measurements was about 16%, such a difference was too small to influence estimates of model parameters.

Table III. The Estimates and Standard Errors of Parameters for the Model of Body Weight Change in Neonates Adopted from (11)

Parameter	Estimate	SE (%RSE)
k_{inbase} (kg/h)	0.00173[a]	
k_{inPNA} (1/h)	0.00479[a]	
k_{outmax} (1/h)	0.00199[a]	
$k_{outbase}$ (1/h)	0.000000482[a,b]	
k_{outPNA} (1/h)	0.0448[a]	
T_{50} (h)	46.3[a]	
H	5.98[a]	
W_0 (kg)	3.61	0.0683 (1.9)
T_{lagV} (h)	48[a]	
T_{lagC} (h)	72[a]	
BW_{0SEX}	− 0.0858	0.0244 (28.4)
BW_{0GA} (1/week)	0.0296	0.00709 (24.0)
$k_{inbaseGA}$ (1/week)	0.103[a]	
$\omega_{kinbase}^2$	0.0841[a] (29%)	
$\omega_{koutmax}^2$	0.01[a] (10%)	
$\omega_{koutbase}^2$	0.64[a] (80%)	
ω_{T50}^2	0.0441[a] (21%)	
ω_{BW0}^2	0.00962 (9.8%)	0.00191 (19.8)
ω_{TlagV}^2	0.01[a] (10%)	
ω_{TlagC}^2	0.01[a] (10%)	
σ_{BW}^2	0.0181	0.00218 (16.1)

[a] Parameter was fixed
[b] $k_{outbase}$ was implemented in NONMEM control stream as 0.0001·theta

A question of interest is to what extent our findings might apply to other drugs used for treatment of neonates. Although many drugs have PK parameters that change with time, our findings will only apply to covariates with time scale relevant to the duration of the specific PK study considered. If a covariate of a patient changes insignificantly between the first and the last sampling time, then any missing value does not impact estimates of PK parameters. For example, propofol clearance has been reported to be positively correlated with a neonate postmenstrual age, but not weight (25). Since the time scale for PMA is a week, missing PMA values in a propofol PK study that lasts less than a week are irrelevant.

The discussed methods of handling missing data assume implicitly that BWs are missing completely at random. According to our study design, BW was measured only at birth and at first dose which implies that the mechanism underlying missing BWs is determined by observation times and therefore is not related to BWs (observed and unobserved). By definition, missing BWs in this case can be classified as data missing completely at random (16,19).

We presented an approach for handling time-dependent missing covariates that requires a dynamic model to account for missing data. Modeling covariates constitutes the basis of FREM that considers them as observations subject to random effects (23). While most applications of FREM are focused on covariate selection, its predictive ability makes it an attractive tool for handling missing covariates. In the case of time invariant covariates, FREM describes the covariate relationships in terms of fixed effects and between-subject variability of covariates as random effects. Time dependence of covariates adds additional complexity to FREM as time courses require fixed effects models. Consequently, the covariate part of FREM becomes a regular time-dependent variable. This is the case for the BW covariate for the APAP population model. A dynamic model for BW that was merged into a FREM is actually more complex than the original PK model. Given that IMPUT and LIKELIHOOD also require modeling BW data and none of the tested methods outperforms one another, selection of FREM for handling missing BWs is recommended, based on evidence collected in this particular analysis. Further studies of populations with missing time-variant covariates are necessary to substantiate our recommendation. Although in the time-dependent covariate BW discussed here, we employed an existing model of BW in term neonates, this might not be the case for other systems with time-dependent covariates. As covariate observations in FREM are considered on par with primary observations, a necessary requirement is to develop a joint model for both.

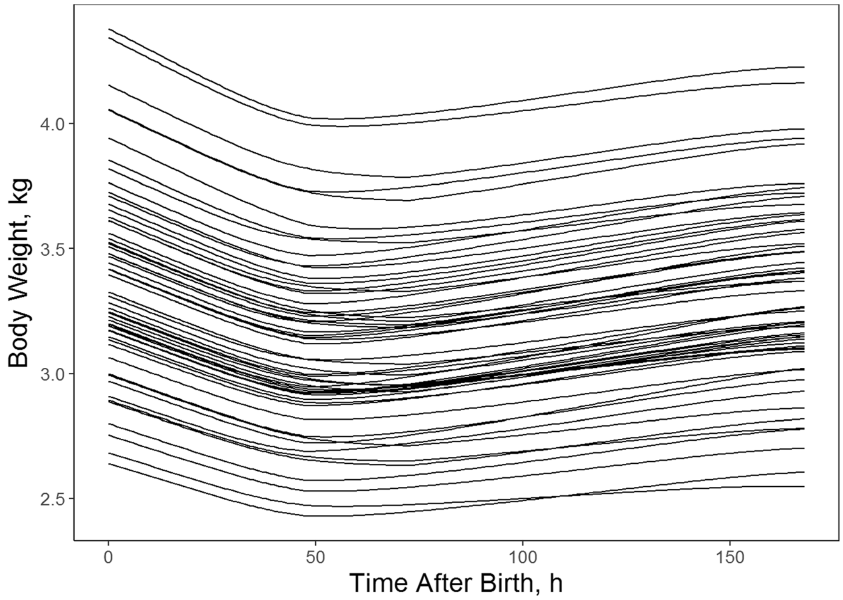


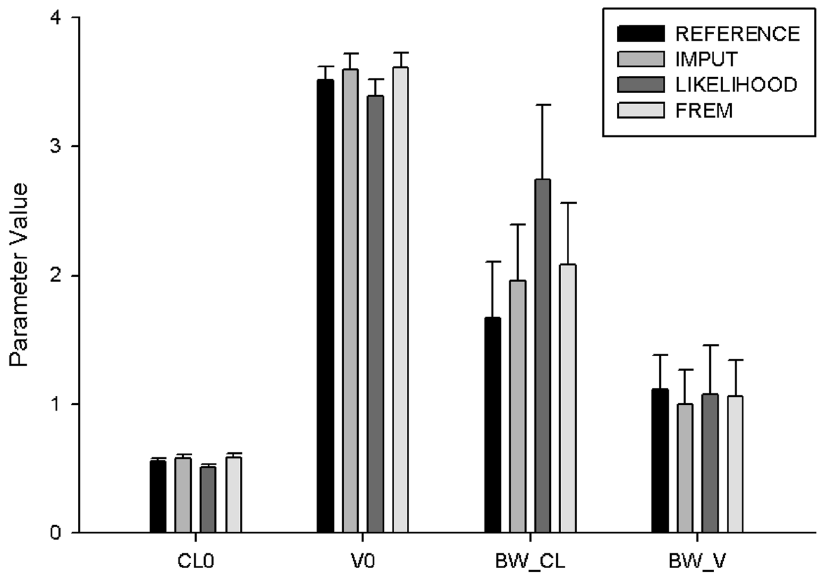
Fig. 2. Simulated time courses of body weight during the first week of life for individual patients in the studied population. The population parameter values used for simulations are shown in Table III

This imposes an additional challenge for the FREM-based population analysis that might make it less attractive than other empirical approaches of handling missing time-dependent data (e.g., last observation carried forward).

For population PKPD models, PK variables can be considered as time-dependent covariates describing PD responses. In the sequential approach, a population PK model is developed first using PK data alone (24). Subsequently, several strategies can be used to analyze PD data. The individual PK parameters (IPP) approach uses maximum a posteriori estimates of individual parameters to describe individual time courses of PK variables deriving individual PD responses. As the PK observations are not used for PD parameter estimation, they can be considered missing and the IPP method is equivalent to IMPUT applied to time-dependent PK covariates. The population PK parameters (PPP) approach fixes the population means of the PK parameters at estimated values obtained from the population PK analysis and corrects the likelihood function for missing PK observations. As such, the PPP method is equivalent with the LIKELIHOOD method discussed in this report. For the population PK parameters and data (PPP&D) approach, the population means of the PK parameters are fixed at values estimated by the population PK model, and the PK observations are modeled jointly with PD observations. PPP&D is similar to FREM as both methods fit jointly covariates and responses. However, FREM differs from PPP&D as it estimates population means for the covariate parameters simultaneously with population means of the response parameters. Our conclusions preferring FREM for handling

Table IV. The Estimates of Parameters and Standard Errors for the Population Models IMPUT, LIKELIHOOD, and FREM. The Remaining FREM Parameters Were Fixed at Values Shown in Table III

Parameter	IMPUT		LIKELIHOOD		FREM	
	Estimate	SE (%RSE)	Estimate	SE (%RSE)	Estimate	SE (%RSE)
CL_0 (L/h)	0.581	0.0280 (4.8)	0.512	0.0238 (4.6)	0.586	0.0297 (5.1)
V_0 (L)	3.60	0.115 (3.2)	3.39	0.126 (3.7)	3.61	0.117 (3.2)
BW_{CL}	1.96	0.430 (21.9)	2.74	0.578 (21.1)	2.08	0.479 (23.0)
BW_V	1.00	0.264 (26.4)	1.07	0.385 (36.0)	1.06	0.279 (26.3)
W_0 (kg)	NA	NA	NA	NA	3.61	0.0657 (1.8)
BW_{0SEX}	NA	NA	NA	NA	−0.0872	0.0252 (28.9)
BW_{0GA} (1/week)	NA	NA	NA	NA	0.0290	0.00740 (25.5)
ω_{CL}^2	0.0945 (30.7%)	0.0178 (18.7)	0.0439 (21.0%)	0.0351 (80.0)	0.0880 (29.7%)	0.0173 (19.7)
ω_V^2	0.0340 (18.4%)	0.00691 (20.3)	0.0324 (18.0%)	0.00990 (30.6)	0.0326 (18.1%)	0.00713 (21.9)
ω_{BW0}^2	NA	NA	NA	NA	0.0858 (29.3%)	0.00197 (2.3)
σ_C^2	0.0270(16.4%)	0.00362(13.4)	0.0268 (16.4%)	0.00366 (13.7)	0.0268 (16.4%)	0.00366 (13.7)
σ_{BW}^2	NA	NA	NA	NA	0.0326	0.00466 (14.3)



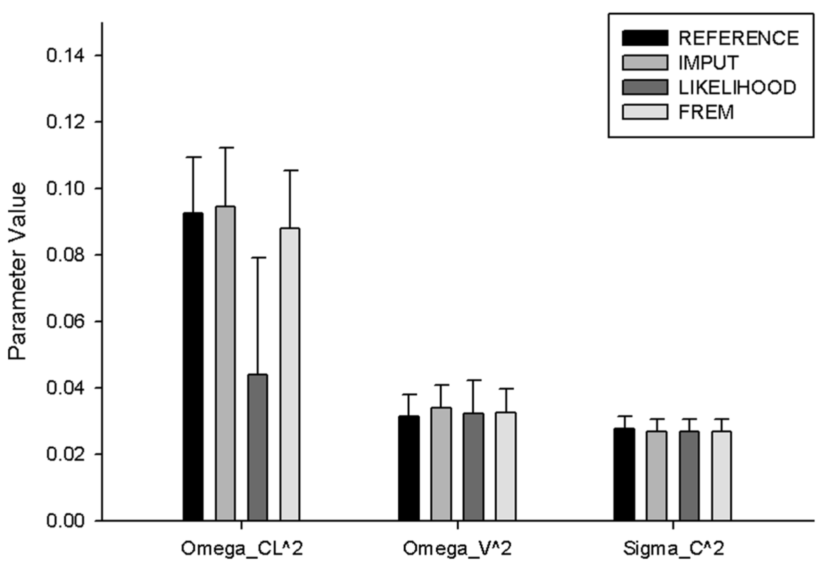


Fig. 3. Estimates of fixed effect parameters CL_0, V_0, BW_{CL}, BW_V grouped by parameter for the studied models: reference, IMPUT, LIKELIHOOD, and FREM. Bars represent the estimated values and the whiskers represent standard errors

data with missing time-dependent covariates coincide with conclusions favoring PPP&D over PPP and IPP for population PKPD data analysis (24).

One of the advantages of using a model for handling missing covariates is that the model does not have to be developed for a population from which the PK data are obtained from. As long as the PK patient population satisfies the conditions under which the covariate model is valid, one can apply it without concerns of model misspecification. This approach allows for borrowing information gathered from one study and applying it to another, resulting in increased effectiveness and reduction of study duration. However, when two patient populations do not entirely overlap, a concern should be raised related to what extent the borrowed information biases the information inferred from the combined models. In our study, the model for BW dynamics was developed for healthy term breastfed neonates, whereas the APAP was administered to preterm and term neonates with a clinical indication for intravenous analgesia who were admitted to intensive care units. Such patients had body weights lower than the range observed for healthy newborns, with 34% of subjects' BW less than the cutoff of 3.2 kg. Although the trends of their BW time courses resembled those of the healthy ones, the factors underlying the weight gain and weight loss (e.g., loss of body fluids and fat, food intake) might differ between these two populations, leading to a bias in BW model parameters. Our approach of censoring the low BW patients from analysis partially alleviated this problem. However, this reduced the variability of observed BWs in our studied patient population and resulted in a potentially lower impact of missing covariates on estimates of the IIV parameters.

In summary, we attempted to assess if missing BWs of neonates receiving APAP can bias estimates of population PK model parameters. The missing covariates were

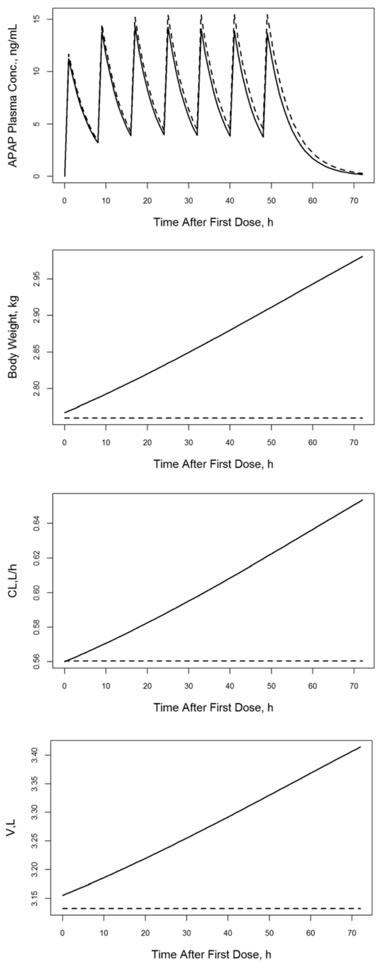


Fig. 4. Simulated time courses of APAP plasma concentration, body weight, clearance, and volume of distribution of a representative subject who received the first APAP dose 3 days after birth predicted by the reference model (dashed lines) and FREM (solid lines)

accounted for by three methods stemming from a dynamic population model of BW change during the first week of life of term neonates. The PK parameters clearance and volume of distribution were insensitive to missing BWs. However, missing BWs affected the covariate relationship parameters, particularly the one describing dependence of clearance on BW. Missing BWs did not inflate estimates of between-subject variability of PK parameters nor impact the predictive performance of the model. None of the tested methods of handling missing covariates was superior to the other, but FREM was the easiest to implement. Our analysis shows that a modeling approach towards handling missing covariates allows borrowing information gathered in various studies as long as they target the same population. This approach is particularly useful for handling time-dependent missing covariates.

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APPENDIX 1. THE LIKELIHOOD FUNCTION FOR IMPUT APPROACH

Given the independence of random variables ε_{Cij}, η_{CLj}, η_{Vj}, the likelihood function of observing APAP plasma concentrations $\boldsymbol{C} = \{C_{ij}\}_{1 \le i \le N, 1 \le j \le n_i}$ at times $\boldsymbol{t} = \{t_{ij}\}_{1 \le i \le N, 1 \le j \le n_i}$ with imputed body weights $\boldsymbol{BW_m} = \{BW_{ij}\}_{1 \le i \le N, 1 \le j \le m_i}$ is of the following form (28):

$$p(\boldsymbol{C}; \boldsymbol{BW_m}, \boldsymbol{\theta}, \boldsymbol{\gamma}, \boldsymbol{t}) = \prod_{i=1}^{N} \int_{\mathbb{R}^2} \prod_{j=1}^{n_i} \varphi\left(C_{ij}; \frac{A(t_{ij})}{V(t_{ij})}, \sigma_C^2\right) \Phi_2(\boldsymbol{\eta}; \boldsymbol{0}, \boldsymbol{\Omega}) d\boldsymbol{\eta} \tag{14}$$

where $\boldsymbol{\theta} = (CL_0, V_0, BW_{CL}, BW_V)$ and $\boldsymbol{\gamma} = (\sigma_C^2, \omega_{CL}^2, \omega_V^2)$ are vectors of model parameters. The function φ describes the normal distribution

$$\varphi(y; \mu, \sigma^2) = \frac{1}{\sqrt{2\pi\sigma^2}} exp\left(-\frac{(y-\mu)^2}{2\sigma^2}\right) \tag{15}$$

and Φ is a probability density function for the multivariate normal distribution:

$$\Phi_2(\boldsymbol{\eta}; \boldsymbol{0}, \boldsymbol{\Omega}) = \left(\frac{1}{\sqrt{2\pi}}\right)^2 \frac{1}{\sqrt{\det \boldsymbol{\Omega}}} exp\left(-\frac{1}{2} \boldsymbol{\eta}' \boldsymbol{\Omega}^{-1} \boldsymbol{\eta}\right) \tag{16}$$

and $\boldsymbol{\Omega} = \mathbf{diag}(\omega_{CL}^2, \omega_V^2)$.

APPENDIX 2. THE LIKELIHOOD FUNCTION FOR LIKELIHOOD APPROACH

Let for subject i^{th} $1 \le j \le m_i$ index times for which BWs are measured and $m_i + 1 \le j \le n_i$ index times for missing BWs. Also, let $1 \le i \le N_V$ be reserved for neonates born by vaginal delivery, and $N_V + 1 \le i \le N$ for neonates born by cesarean delivery. Then, the likelihood function of observing APAP plasma concentrations becomes (26):

$$(\boldsymbol{C}; \boldsymbol{BW}, \boldsymbol{\theta}, \boldsymbol{\gamma}, \boldsymbol{t}) = \prod_{i=1}^{N} \int_{\mathbb{R}^2} \prod_{j=1}^{m_i} \varphi\left(C_{ij}; \frac{A(t_{ij})}{V(t_{ij})}, \sigma_C^2\right) \Phi_2(\boldsymbol{\eta}; \boldsymbol{0}, \boldsymbol{\Omega}) d\boldsymbol{\eta} \cdot$$

$$\prod_{i=1}^{N} \int_{\mathbb{R}^9} \prod_{j=m_i+1}^{n_i} \varphi\left(C_{ij}; \frac{A(t_{ij})}{V(t_{ij})}, \sigma_C^2\right) \Phi_9(\boldsymbol{\eta}; \boldsymbol{0}, \boldsymbol{\Omega_C}) d\boldsymbol{\eta} \tag{17}$$

Here, $\boldsymbol{BW} = \{BW_{ij}\}_{1 \le i \le N, 1 \le j \le m_i}$ denotes the observed BWs, and

$$\boldsymbol{\Omega_C} = \mathbf{diag}\left(\omega^2_{CL}, \omega^2_V, \omega^2_{kinbase}, \omega^2_{koutmax}, \omega^2_{koutbase}, \omega^2_{T50}, \omega^2_{BW0}, \omega^2_{TlagC}, \omega^2_{TlagV}\right)$$

Only model parameters $\boldsymbol{\theta}$ and $\boldsymbol{\gamma}$ were estimated, while the fixed effect parameters for the BW model and the entries of $\boldsymbol{\Omega_C}$ other than $\omega^2_{CL}, \omega^2_V$ were fixed.

Appendix 3. The Likelihood Function for FREM Approach

The joint likelihood function for observed APAP plasma concentrations $\boldsymbol{C} = \{C_{ij}\}_{1 \le i \le N, 1 \le j \le n_i}$ and observed BWs $\boldsymbol{BW} = \{BW_{ij}\}_{1 \le i \le N, 1 \le j \le m_i}$ becomes:

$$p(\boldsymbol{C}, \boldsymbol{BW}; \boldsymbol{\theta_F}, \boldsymbol{\gamma_F}, \boldsymbol{t}) = \prod_{i=1}^{N} \int_{\mathbb{R}^2} \prod_{j=1}^{m_i} \varphi\left(C_{ij}; \frac{A(t_{ij})}{V(t_{ij})}, \sigma^2_C\right) \Phi_2(\boldsymbol{\eta}; \mathbf{0}, \boldsymbol{\Omega}) d\boldsymbol{\eta} \prod_{i=1}^{N} \int_{\mathbb{R}^7} \prod_{j=1}^{m_i} \varphi\left(BW_{ij}; BW(t_{ij}), \sigma^2_{BW} BW(t_{ij})^2\right) \Phi_7(\boldsymbol{\eta}; \mathbf{0}, \boldsymbol{\Omega_W}) d\boldsymbol{\eta} \quad (18)$$

where

$$\boldsymbol{\theta_F} = \left(CL_0, V_0, BW_{CL}, BW_V, k_{inbase}, k_{inPNA}, k_{outmax}, k_{outbase}, k_{outPNA}, k_{inbaseGA}, T_{50}, H, W_0, T_{lagV}, T_{lagC}, BW_{0SEX}, BW_{0GA}\right)$$

and

$$\boldsymbol{\gamma_F} = \left(\sigma^2_C, \sigma^2_{BW}, \omega^2_{CL}, \omega^2_V, \omega^2_{kinbase}, \omega^2_{koutmax}, \omega^2_{koutbase}, \omega^2_{T50}, \omega^2_{BW0}, \omega^2_{TlagC}, \omega^2_{TlagV}\right)$$

are vectors of model parameters. Here

$$\boldsymbol{\Omega_W} = \mathbf{diag}\left(\omega^2_{kinbase}, \omega^2_{koutmax}, \omega^2_{koutbase}, \omega^2_{T50}, \omega^2_{BW0}, \omega^2_{TlagC}, \omega^2_{TlagV}\right)$$

Only CL_0, V_0, BW_{CL}, BW_V, W_0, BW_{0SEX}, BW_{0GA}, and $\sigma^2_C, \sigma^2_{BW}, \omega^2_{CL}, \omega^2_V, \omega^2_{BW0}$ were estimated, while the remaining model parameters were fixed.

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