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ORIGINAL ARTICLE

Modeling Interindividual Variability in Physiologically Based Pharmacokinetics and Its Link to Mechanistic Covariate Modeling

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Covariate modeling is a key step in the analysis of clinical data and is essential for establishing dosing recommendations for specific populations, e.g., in obese individuals and children. So far, no systematic approach exists to leverage the knowledge inherent in physiologically based pharmacokinetic (PBPK) models in this context. We introduce (i) a novel approach to model interindividual variability in PBPK models based on lean body weight (LBW); and (ii) a systematic approach to translate interindividual variability into the design of mechanistic covariate models. We derive a new covariate relation for the volume of distribution at steady state (V_{ss}) that seamlessly integrates body weight and LBW as covariates, with a weighting factor depending on the physicochemical properties of the drug. We further show that for children, PBPK-based extrapolation and allometric scaling result in very similar predictions for V_{ss} and blood clearance.
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Analyzing interindividual variability in pharmacokinetics (PK) and understanding its origins is of critical importance in clinical development.[1–3] Typically, clinical PK data are analyzed in a population analysis framework aiming to investigate the impact of covariates (independent variables that possibly explain interindividual variability) on PK parameters to explain observed interindividual variations. The development of covariate models is generally a time-consuming and nontrivial task.[4] Although most covariate models are likely to be motivated by mechanistic or (patho)physiological considerations, a formal derivation from underlying mechanistic principles is rarely presented. An approach that supports the systematic derivation of covariate models based on detailed mechanistic knowledge is therefore highly desirable. In drug development, typically whole-body physiologically based pharmacokinetic (PBPK) models are used to represent drug PK in a mechanistic way.[5,6]

The objectives of this article are to (i) develop a scaling approach for anatomical and physiological parameters used in PBPK models of humans incorporating the influence of important covariates; and (ii) develop a systematic approach to translate this interindividual variability into the design of mechanistic covariate models. We expect clinical drug development to benefit from our approach by generating expectations on the size of interindividual variations for a given study population. Observed interindividual variability for given clinical data can then be compared with the expected variations, thereby enabling one to gain further insight into the diverse underlying sources of observed variations.

In an earlier study we introduced the link between PBPK models and classical compartment models for a reference individual based on the concept of lumping.[7] Taking into account interindividual variability in anatomical and physiological parameters, the lumping approach allowed us to translate these variations to the level of classical compartment models. We obtained mechanistically justified covariate models. Although there exist various sources of interindividual variability, in this study we focused on the predictable impact of variations in anatomical and physiological parameters linked to covariates. Our approach, however, is equally applicable to other sources of interindividual variations, including characteristics of the disease state. In the literature, several approaches to model interindividual variations in physiological parameters in PBPK models have been proposed.[8–12] The most relevant approaches, however, allowed only poorly to reproduce experimentally observed interindividual variability. In comparison, the herein proposed lean body weight (LBW) scaling approach predicts much more realistic variations.

RESULTS

LBW-scaling approach best predicts interindividual variability in organ weights

We propose a new approach to scale anatomical and physiological parameters that takes into account important anthropometric characteristics like body weight (BW), LBW, body height (BH), body mass index (BMI), and body surface area (BSA). It can be interpreted as a size model approach[13] for anatomical and physiological data, where reference values

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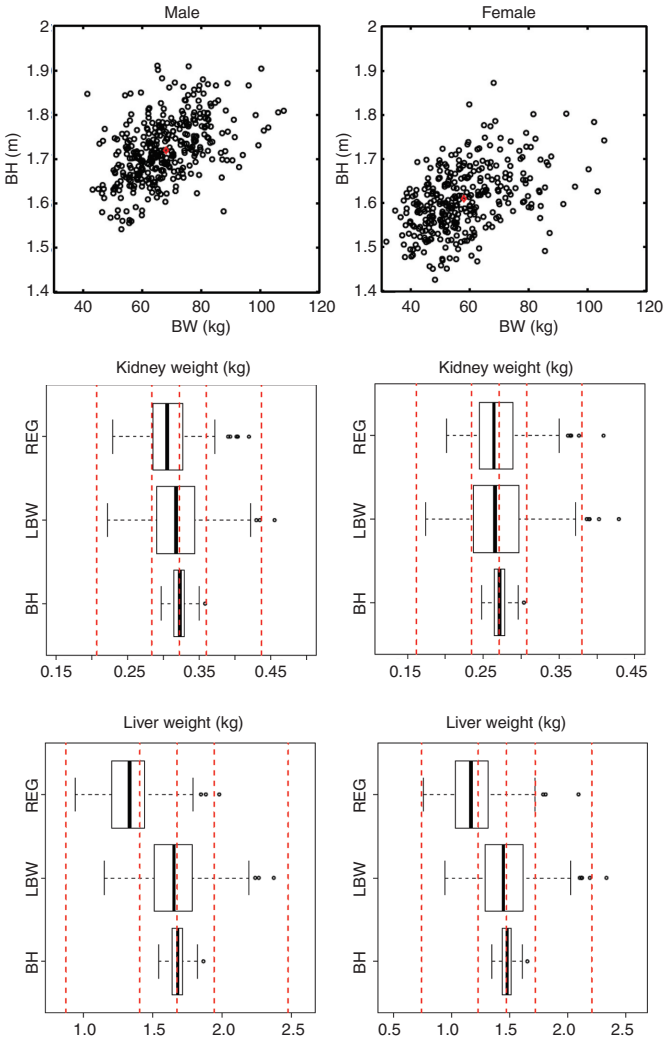

Figure 1 Distribution of body height (BH) and body weight (BW) for a male (left) and female (right) (populations characteristics given in Supplementary Table S2 online (red diamond = mean value)). Comparison of lean body weight (LBW) scaling approach, regression equation approach (REG), and BH scaling approach (given in terms of box plots) in comparison with experimental data (characterized in terms of red dashed lines) from the autopsy study[14] for male (left) and female (right) population. Box plots and dashed lines from left to the right: the lower whisker, the 0.25 quartile, the median, the 0.75 quartile, and the upper whisker.

are scaled with a factor incorporating relevant anthropometric characteristics to obtain parameter values of the target individual (see section "Interindividual variations of anatomical and physiological factors used in whole-body PBPK models"). **Figures 1** and **2** show the predicted distribution of organ weights based on the herein proposed LBW-scaling approach in comparison with existing methods (BH-scaling[9] and the regression equation approach[10]) and experimental data from a large autopsy study by de la Grandmaison *et al.*[14] For heart, kidneys, liver, and lung, the LBW-scaling approach showed closest agreement with the experimental data. The BH-scaling approach largely underestimated the variability, whereas the regression equation approach poorly predicted mean values. For spleen, the LBW-scaling approach largely underestimated observed variability—in comparison with the other two approaches, however, it still generated more realistic variations.

Variations in partition coefficients contribute substantially to variability of predicted blood concentration–time profiles

Interindividual variability of anatomical and physiological parameters contribute to observed variations in venous blood concentrations. In addition, variations in tissue partitioning—specified in terms of tissue-to-blood partition coefficients—can also be expected to contribute to observed variations in drug blood concentrations. As can be inferred from **Figure 3**, interindividual variations in tissue partitioning do have a substantial impact on venous blood concentrations, potentially even larger than variations due to interindividual variability in tissue volumes and blood flows. When performing predictions based on PBPK models, the variability in tissue partition coefficients should be taken into account. Such variations have been observed experimentally.[15,16] In a population analysis context, variations in

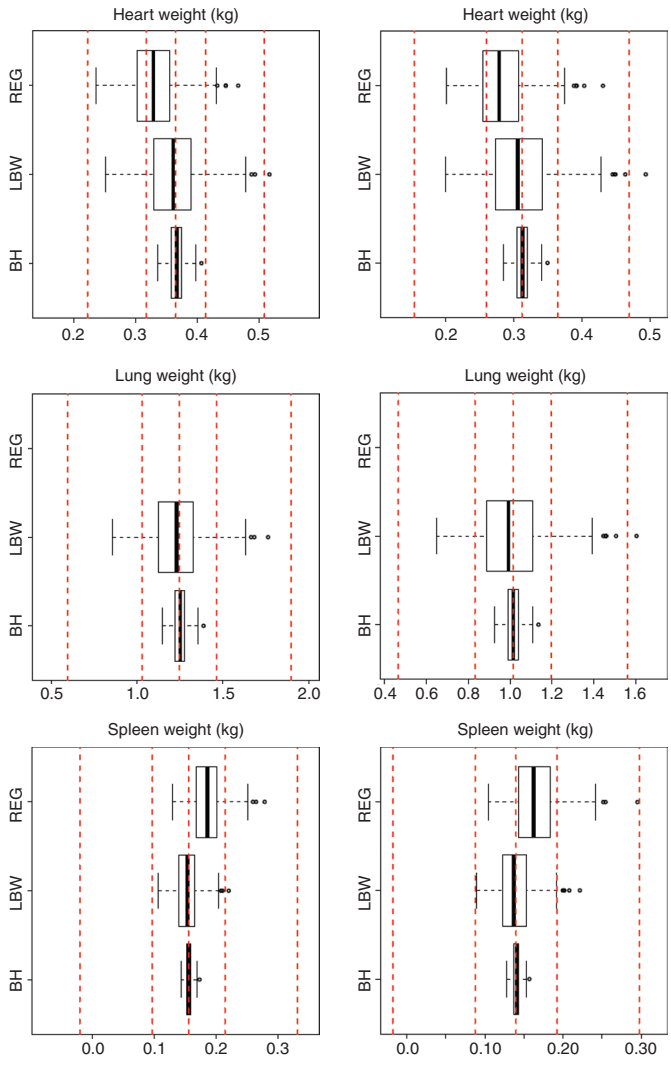

Figure 2 For description, see caption of Fig. 1. For lung, also a lean body weight (LBW) scaling approach is used in ref. 10.

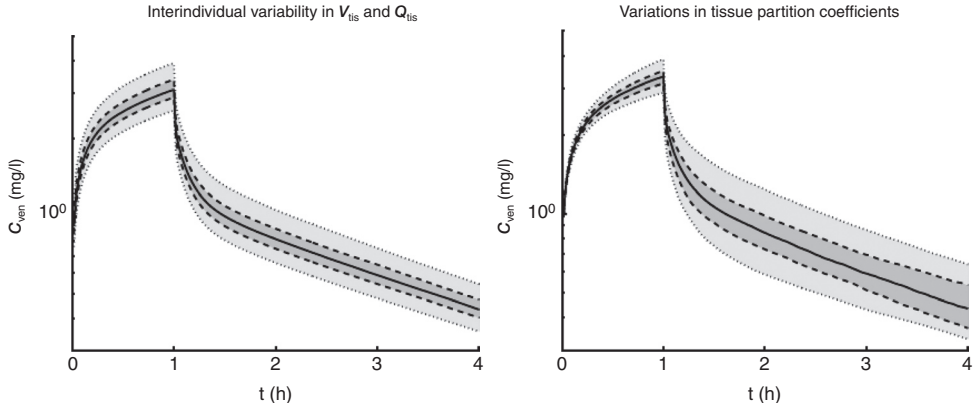

Figure 3 Comparison of the impact of interindividual variability and variations in tissue partitioning on venous blood concentration. Left: variations in blood concentrations solely due to interindividual variability of anatomical and physiological factors as present in the virtual de la Grandmaison male subpopulation ($n = 355$). Right: variations of blood concentration solely due to variations in tissue partition coefficients (for otherwise identical individuals with the mean characteristics of the de la Grandmaison male subpopulation). Distributions show 5, 25, median, 75, 95 percentiles. Predictions based on the generic physiologically based pharmacokinetic model and a 1 h i.v. infusion.

tissue composition most likely translate into unexplained variability.

Mechanistic covariate models capture interindividual variations in anatomical and physiological factors

We propose a new approach to mechanistic covariate modeling that reduces a PBPK model—representing current mechanistic knowledge of the drug's PK and important interindividual variations in anatomical and physiological parameters—to a simple compartment model with mechanistically integrated covariates (described in sections "Mechanistic approach to covariate modeling" and "Generic compartment models with mechanistically integrated covariates"). By construction, this model is consistent with the underlying mechanistic principles of the PBPK model. **Figure 4** compares the venous blood concentrations predicted by the whole-body PBPK and the compartment model with mechanistically integrated covariates for a single individual (left) and a larger virtual population (right). All predictions are in excellent agreement and in accordance with theoretical expectations. They illustrate the predictive power of the new approach to mechanistic covariate modeling.

PBPK extrapolation and allometric scaling give comparable results for predicting volume of distribution and blood clearance in children

Predicting children PK from adult PK data via extrapolation is a common approach. PBPK models extrapolate by replacing adult physiological and anatomical parameter values by the corresponding children values. By contrast, allometric scaling approaches extrapolate by scaling adult pharmacokinetic parameter values to children based on size models.[13] Although children are not small adults from a physiological and anatomical point of view (**Supplementary Tables S3 and S4** online), we aimed at comparing the impact of these physiological and anatomical differences on the PK of a diverse collection of 25 drugs with different physico-chemical properties (Table 3 in ref. 7). **Figure 5** compares the predicted volume of distribution V_{ss} and hepatic blood clearance CL for children based on PBPK extrapolation and allometric scaling. Both approaches gave very similar results, which are as similar or different as are predictions resulting from extrapolation with PBPK models based on different published sets of parameter values for children[9,10] (**Table 1**). Hence, for children aged 5 and older, both approaches can be considered comparable. This can be expected to hold true also for younger children (2–5 years).

DISCUSSION

There are several approaches to model interindividual variations of physiological parameters in PBPK models[8–12,17]; but only few provide all necessary information to predict it. Price *et al.*[10] review various regression equations describing the variations of physiological parameter observed in different studies as a function of different covariates. A general disadvantage of the regression analysis approach is its dependence on the characteristics of the underlying study population. This might be the reason for the large deviation observed for liver and heart weight in **Figure 1**, as the underlying study population was Japanese (liver) and Korean (heart).[18,19] Willmann *et al.*[9] took a different approach restricting their set of covariates essentially to BH and BW and including additional random perturbations to create a virtual population. Adipose tissue weight is defined as the weight that remains after scaling all other tissues. Hence, any error in scaling nonadipose tissues has an impact on the accuracy of adipose weight. The predicted variations by the BH-scaling approach in **Figures 1** and **2** are much lower than the experimentally observed variations. Young *et al.*[12] took an intermediate approach. They fit up to fifth-order polynomials in BW. As in the Willmann *et al.* approach, adipose weight is defined as the difference between BW and the sum of all remaining tissue weights, resulting in the above-mentioned disadvantage.

By contrast, our approach is based on a set of common anthropometric characteristics that have proven to be of relevance in the covariate analyses of clinical trails and that are

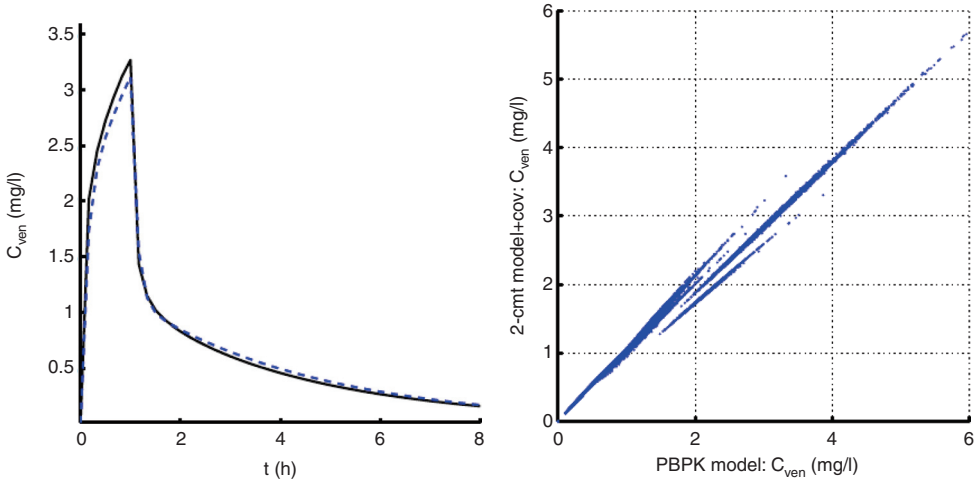

Figure 4 Physiologically-based pharmacokinetic (PBPK) model vs. associated two-compartment model with mechanistically integrated covariates. Prediction of venous blood concentration after administration of 400 mg lidocaine (60 min i.v. infusion). Left: comparison of venous blood concentration–time profile for a single individual based on the PBPK model (solid black) and the two-compartment model (dashed blue). Right: plot based on the virtual de la Grandmaison population with male/female individuals.

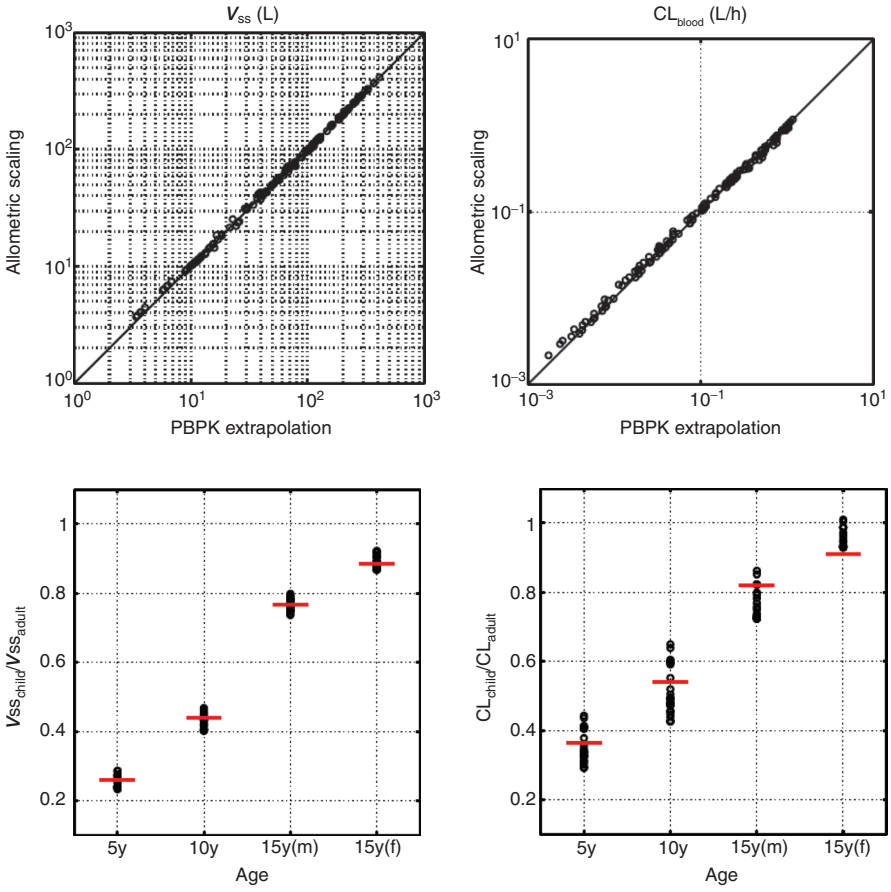

Figure 5 Physiologically-based pharmacokinetic (PBPK) extrapolation vs. allometric scaling for the volume of distribution V_{ss} (left) and the hepatic blood clearance CL (right) for a diversity of 25 drugs with different physico-chemical properties. Top: predicted vs. predicted. Bottom: values normalized by the corresponding adult value (horizontal lines mark allometric scaling value).

Table 1 Comparison of allometric scaling vs. PBPK extrapolation as well as PBPK extrapolations based on alternative published sets of parameter values for children

		5 years	10 years	15 years (m/f)
Allometry vs. PBPK	$\sigma(V_{ss})$	0.07	0.05	0.02/0.02
	σ(CL)	0.1	0.1	0.06/0.03
$PBPK_{alternative}$ vs. PBPK	$\sigma(V_{ss})$	0.08	0.1	0.05/0.09
	σ(CL)	0.9	0.2	0.01/0.03

Differences are reported as SD σ(CL) and $\sigma(V_{ss})$ for blood clearance CL and volume of distribution at steady state V_{ss}, respectively.
PBPK, physiologically based pharmacokinetic.

underpinned by mechanistic considerations. Due to its important role in PK, adipose weight was defined as accurately as possible using its definition. The LBW-scaling approach predicts most realistically the experimentally observed variations in organ weights (**Figures 1** and **2**). The lack of correlation of spleen weight with BW, BH, or BMI has been observed in females,[20] and might be attributable to its "spongy" composition.[10] The presented approach also offers a simple and systematic way of integrating new covariates, like creatinine clearance. Once the functional relationship between the new covariate and the variation of some anatomical or physiological parameter is (reasonably well) established, application of the proposed mechanistic lumping approach results in a theoretically justified covariate model.

Our analysis suggests a new covariate relation for the volume of distribution at steady state V_{ss} that seamlessly integrates both BW and LBW as covariates. Combining equations (26), (27), and (35) yields

$$V_{ss} = V_{ss,ref} \cdot \left((1-R) \cdot \frac{LBW}{LBW_{ref}} + R \cdot \frac{BW - LBW}{BW_{ref} - LBW_{ref}} \right) \qquad (1)$$

where the drug-specific parameter $R = \theta_{adi}/V_{ss,ref}$ denotes the adipose-to-total volume of distribution ratio of the reference individual. This parameter can be estimated from clinical data (described later). For drugs with, e.g., R = 1/2, the above covariate relation implies that doubling of adipose weight would have the same impact on V_{ss} as doubling LBW. Such behavior is associated with large differences in adipose tissue partitioning $\hat{K}_{ad}$ (see equation (21) for definition of the elimination-corrected partition coefficient) compared with tissue partitioning into the remaining tissues $\hat{K}_{tis}$, and has been observed clinically.[21] Of note, not the absolute magnitude of $\hat{K}_{ad}$ is relevant, but its magnitude relative to the other partition coefficients (drugs with $\hat{K}_{adi} \geq 4.2 \cdot \hat{K}_{tis}$ for all tis ≠ adi for the reference male have $R \geq 1/2$, which follows from equations (26) and (27), and V_{tot}/V_{adi} = 67.0/15.8 = 4.2, where the total volume was determined as sum of all tissue volumes excluding

rest of body). For females, this value is even lower, since V_{tot}/V_{adi} = 55.1/20.7 = 2.7).

The above covariate relation integrates a number of observations into a unified quantitative framework: it is remarked that V_{ss} is consistently increased in patients with excess adipose tissue and that this increase at least in part seems to be related to the physico-chemical properties of the drug.[22] Cheymol[23] suggested an approach to compute a loading dose in obese individuals that takes into account whether the drug distributes predominantly into the lean tissue or also into the fat tissue. Anderson and Holford[13,24] introduced the concept of normal fat mass NFM = FFM + Ffat . (BW – FFM), where FFM denotes the fat free mass and Ffat denotes the fat weight fraction that is specific to the drug and pharmacokinetic parameter to be scaled. For V_{ss}, this yields

$$V_{ss} = V_{ss,\,ref} \cdot \left(\frac{\mathrm{FFM} + F_{fat} \cdot (\mathrm{BW} - \mathrm{FFM})}{\mathrm{FFM}_{ref} + F_{fat} \cdot (\mathrm{BW}_{ref} - \mathrm{FFM}_{ref})} \right),$$

which is identical to equation (1) when identifying FFM with LBW and

$$F_{fat} = \frac{R}{1-R} \cdot \frac{\mathrm{LBW}_{ref}}{\mathrm{BW}_{ref} - \mathrm{LBW}_{ref}}.$$

Our seamless LBW and BW-scaling equation (1) integrates all these observations and empirically derived relations into a coherent, mechanistic framework.

For drugs with a small therapeutic window, reliable scaling relations are mandatory for extrapolation. Whether or not these can be obtained from given experimental data also depends on the underlying study design: to this end, we rewrite equation (6) to define the fraction LBW

$$\%\mathrm{LBW} = \frac{\mathrm{LBW}}{\mathrm{BW}} = \frac{M_{BMI}}{K_{BMI} + \mathrm{BMI}}. \qquad (2)$$

The term (1 – %LBW) is related to the percentage body fat, a common measure of obesity.[25–27] We obtain LBW = %LBW·BW and OW_{adi} = BW – LBW = (1 – %LBW) · BW. If the study population only includes volunteers/patients such that (approximately)

$$\mathrm{BMI} = \mathrm{const}, \text{ or equivalently } \%\mathrm{LBW} = \mathrm{const} \qquad (3)$$

then $\mathrm{LBW}/\mathrm{LBW}_{ref}$ = (BW – LBW)/(BW_{ref} – LBW_{ref}) = $\mathrm{BW}/\mathrm{BW}_{ref}$. This is typically the case in phase I studies. In such a case, the scaling equation (1) simplifies to

$$V_{ss} = V_{ss,ref} \cdot \frac{\mathrm{BW}}{\mathrm{BW}_{ref}}. \qquad (4)$$

Most importantly, this equation holds for any value of *R*, i.e., for any drug. For a study population satisfying condition (3), scaling with BW is equally appropriate, and—depending on the actual clinical data—might even result in the best covariate relation estimated from the data. BW-based scaling, however, could be completely misleading, when *R* is indeed large. In this case, extrapolation to obese based on BW would result in erroneous predictions. If precise scaling is mandatory, the design of a clinical study including volunteers with a range of %LBW values is expected to be more informative. In this context, preclinical data may be exploited to generate expectations. For example, one study found a prolonged accumulation of diazepam in obese individuals[21]. Elimination half-life was greatly prolonged in the obese subjects (82 vs. 32 h), with no change in total metabolic clearance. Instead, a large increase in volume of distribution (228 vs. 70 liters) was the reason for prolongation of elimination half-life.[21] Preclinical rat data[28] on tissue-to-plasma partition coefficients show a marked difference with $K_{adi} \geq 4.8 \cdot K_{tis}$, for tis ≠ adi (no experimental data for bone were available). Hence, translating data from rat to human would have at least flagged this issue. This knowledge is important for the appropriate design of clinical trials, following the spirit of the "learning vs. confirming" approach.

We finally remark that the proposed approach allows one to estimate the adipose tissue-to-blood partition coefficient K_{adi} *in vivo* based on estimated values of *R* and $V_{ss,\,ref}$.

Tissue-to-blood partition coefficients K_{tis} are key parameters in PBPK models. We predicted K_{tis} based on the tissue-to-unbound plasma partition coefficient Ku_{tis}.[28,29] For rat, the predicted accuracy is reported to be 84–89% within a factor 3 of experimental values.[28–30] Although part of this discrepancy is expected to be related to the "simplicity" of the *in silico* model to predict Ku_{tis} (taking into account only the most relevant interactions), we believe that a relevant part is related to interindividual variability in tissue composition, as has been observed experimentally.[15,16] The impact of such variations might be as relevant as variations in tissue volumes and blood flows (**Figure 3**). PBPK predictions incorporating the covariates BW and LBW, and variations in K_{tis} can generate expectations about the relative impact of each of these parameters-a valuable information when judging the plausibility of covariate modeling results.

Our PBPK extrapolation approach to children is comparable with existing approaches.[31–33] Our analysis considered only children of 5, 10, and 15 years of age. Similar results might be expected for children of 2 years of age. For children younger than 2 years, maturation processes also have to be taken into account.[13,17,31,34] The PBPK extrapolation approach and allometric scaling to children performed very similarly. This is in line with previous findings.[35] Most notably, the difference between the extrapolation to children based on two PBPK models (using different anatomical and physiological parameter values) is of the same size as the difference between allometric scaling and PBPK extrapolation. From this perspective, both approaches can be considered interchangeable.

In this study, we focused on modeling interindividual variability in anatomical volumes and physiological blood flows. Many more factors are known to influence drug PK. Regarding the potential impact of hepatic and renal clearance, potential factors are discussed in ref. 36. Anderson and Holford[13] argue, however, that renal clearance is not increased in obese individuals and it would be reasonable to suppose that FFM is a good predictor of clearance because fat is not a clearance organ and is unlikely to be a determinant of elimination function. In addition, we did not consider maturation processes that are important for the extrapolation to infants (babies up to the age of 2 years). We believe, however, that the presented approach will also be of use to address these questions.

The Matlab files of the study are available as **Supplementary Data** online.

THEORY

A general introduction to PBPK modeling can be found in refs. 5 and 6. In the sequel, we used the term "tissue" (subscript "tis") to jointly refer to tissues and organs. As it is commonly done for PBPK models of small molecule drugs, we assumed that the drug distributes within the body via advection of the blood flow and via passive diffusion homogeneously into tissues, and that the drug is eliminated predominantly by the liver. In the presence of additional or more specific information, the PBPK model can be adapted accordingly, e.g., to account for gut metabolism, renal excretion, etc. This also includes pathophysiological and alterations in patients.

Interindividual variations of anatomical and physiological factors used in whole-body PBPK models

Individual parameters like tissue volumes V_{tis}, blood flows Q_{tis}, and tissue partition coefficients K_{tis} were predicted from parameter values of a reference individual based on scaling factors:

$$V_{tis} = SV_{tis} \cdot V_{tis,ref}, \quad Q_{tis} = SQ_{tis} \cdot Q_{tis,ref}, \quad K_{tis} = SK_{tis} \cdot K_{tis,ref}$$

where SV_{tis}, SQ_{tis}, and SK_{tis} denote the scaling factors for tissue weights, blood flows, and partition coefficients, respectively, and 'ref' refers to the reference individual. Parameter values for the reference individuals (children of age 5, 10, 15 years, and adults age 20–50 years) are listed in the **Supplementary Data** (A.2) online. Thereby, age is included as a (categorical) covariate.

The input of our approach are all parameter values with an index 'ref', including anatomical and physiological data as well as drug specific data. In addition, the covariate values of the reference as well as the target individual are required.

All parameters and covariates are based on units (m) for length, (kg) for weight, (L) for volume and (min) for time, unless otherwise stated.

For many drugs, the adipose tissue is a key distributional space.[7] We determined organ weight (OW) of the adipose tissue by

$$OW_{adi} = BW - LBW. \tag{5}$$

In the absence of knowledge of LBW, it was approximated by fat free mass FFM,[37,38] i.e.,

$$LBW \approx FFM = \frac{M_{BMI}}{K_{BMI} + BMI} \cdot BW, \tag{6}$$

with M_{BMI} = 9,270/216 and K_{BMI} = 6,680/216 (for male) and M_{BMI} = 9,270/244 and K_{BMI} = 8,780/244 (for female), and body mass index defined as

$$BMI = BW/BH^2. \tag{7}$$

We obtained the following scaling factor for adipose volume

$$SV_{adi} = \frac{BW - LBW}{BW_{ref} - LBW_{ref}}. \tag{8}$$

We assumed that the brain volume is constant in each age class, i.e.,

$$SV_{bra} = 1 \tag{9}$$

consistent with ref. 39 In line with ref. 10, we considered body surface area as a factor of proportionality for the skin tissue, resulting in

$$SV_{ski} = \frac{BSA}{BSA_{ref}}. \tag{10}$$

In the absence of knowledge of BSA, we used the approximate formula[40]:

$$BSA = \sqrt{\frac{BH \cdot BW}{36}}. \tag{11}$$

For the remaining tissues we assumed that all tissues scale identically with scaling factor

$$SV_{tis} = \frac{LBW - OW_{bra} - OW_{ski}}{LBW_{ref} - OW_{bra,ref} - OW_{ski,ref}}, \tag{12}$$

exploiting $BW - (OW_{adi} + OW_{bra} + OW_{ski}) = LBW - OW_{bra} - OW_{ski}$. In the **Supplementary Data** (A.4) online we provided supportive evidence for scaling cardiac output according to

$$SQ_{co} = SV_{tis}. \tag{13}$$

Tissue blood flows were then scaled by assuming $SQ_{tis} = SQ_{co}$. Using the fraction unbound in plasma fuP, the blood-to-plasma ratio BP and equation (49) (**Supplementary Data** online), we obtained

$$K_{tis} = \frac{fuP \cdot BP_{ref}}{BP \cdot fuP_{ref}} \cdot K_{tis,ref} \tag{14}$$

resulting in

$$SK = \frac{fuP}{fuP_{ref}} \cdot \frac{BP_{ref}}{BP}. \tag{15}$$

The intrinsic hepatic clearance CL_{int} was assumed to scale with the liver volume

$$CL_{int} = V_{liv} \cdot \frac{CL_{int,ref}}{V_{liv,ref}} = SV_{liv} \cdot CL_{int,ref}. \tag{16}$$

Exploiting $SQ_{liv} = SV_{liv}$ yielded for the hepatic extraction ratio E_{hep} (based on equation (52), **Supplementary Data** online)

$$E_{hep} = \frac{SK \cdot CL_{int,ref} \cdot K_{liv,ref}}{SK \cdot CL_{int,ref} \cdot K_{liv,ref} + Q_{liv,ref}} \tag{17}$$

and the hepatic blood clearance $CL_{blood} = E_{hep} SQ_{liv} Q_{liv,ref}$.

Mechanistic approach to covariate modeling

Starting point is a PBPK model whose anatomical and physiological parameters incorporate important anthropometric characteristics via some scaling approach. It then proceeds in two steps:

- Derivation of a low-dimensional mechanistic compartment model and parameterization by reduction of the PBPK model via lumping.
- Derivation of the final covariate model from the scaling relation of the lumped parameters.

By design, the proposed procedure guaranteed consistency of the mechanistic low-dimensional compartment model with the original whole-body PBPK model.

Derivation of a low-dimensional compartment model and parameterization by reduction of the PBPK model via lumping. The reduction of the whole-body PBPK model was based on the lumping approach described in ref. 7, which lumps tissues with similar kinetics. This results typically in one- or two-compartment models with lumped volumes V_L, lumped blood flows Q_L, lumped tissue-to-blood partition coefficients K_L, and hepatic blood clearance CL_{blood}. On the basis of the scaling relations (5)–(17) and the lumping approach,[7] parameters of the lumped model were given as

$$V_L = \sum_{tis} SV_{tis} \cdot V_{tis,ref} \tag{18}$$

$$K_L = \frac{1}{V_L} \sum_{tis} SV_{tis} \cdot V_{tis,ref} \cdot SK \cdot \hat{K}_{tis} \tag{19}$$

$$Q_L = \sum_{tis} SQ_{co} \cdot Q_{tis,ref}, \tag{20}$$

where the sum is taken over all tissues that are grouped together in the lumped compartment 'L'. Above, $\hat{K}_{tis}$ denotes the elimination-corrected tissue-to-blood partition coefficient

$$\hat{K}_{tis} = (1 - E_{tis}) K_{tis} \tag{21}$$

where E_{tis} denotes the tissue-specific extraction ratio (see ref. 7 for details). In our generic PBPK model, it is $E_{liv} = E_{hep} > 0$ and $E_{tis} = 0$ for all other tissues. For artery and vein, we formally set $\hat{K}_{ven} = \hat{K}_{art} = 1$.

Derivation of the final covariate model from the scaling relation of the lumped parameters. If tissue volumes were all scaled with identical SV = SV_{tis}, then equation (18) could be simplified to

$$V_L = \sum_{tis} SV_{tis} \cdot V_{tis,ref} = SV \cdot \sum_{tis} V_{tis,ref} = SV \cdot V_{L,ref}. \tag{22}$$

Thus, the individual volume V_L could be derived from the reference volume $V_{L,ref}$ via the simple scaling relation $V_L = SV \cdot V_{L,ref}$. In general, however, volume scaling factors might differ between tissues. To bridge the gap to the simplicity of common empirical covariate relations, we finally chose an approximate scaling factor so that equation (22) holds approximately.

For LBW-scaling, we defined the common scaling factors as

$$SF_{LBW} = \frac{LBW}{LBW_{ref}} \quad \text{and} \quad SF_{adi} = \frac{BW - LBW}{BW_{ref} - LBW_{ref}}, \tag{23}$$

For all tissues except brain and skin, SF_{LBW} provides an excellent approximation to the scaling factors SV_{tis} in equation (12), as brain and skin jointly comprise only 8% of LBW (reference adult). For brain and skin, the approximation has to be seen in the light of equation (22). Again, as brain and skin comprise only 8% of LBW, the error introduced to the lumped volume V_L in equation (22) is negligible. Regarding the blood flows, we also approximate the scaling factor SQ by SF_{LBW} in view of equation (13). Finally, we approximate SK = 1 in equation (15).

Generic compartment models with mechanistically integrated covariates

We illustrate the mechanistic approach to covariate modeling to derive a two-compartment models with mechanistic covariate models that are consistent with the underlying PBPK model and interindividual variability based on the LBW-scaling approach.

For the generic situation that the liver is lumped into the central compartment and the adipose tissue is part of the peripheral compartment (see ref. 7 for details), we obtained

$$V_1 \frac{d}{dt} C_1 = Q(C_2 - C_1) - CL_{blood} \cdot C_1 + r_{admin} \tag{24}$$

$$V_2 \frac{d}{dt} C_2 = Q(C_1 - C_2) \tag{25}$$

with mechanistic covariate models

$$V_1 = \Theta_1 \cdot SF_{LBW} \tag{26}$$

$$V_2 = \Theta_2 \cdot SF_{LBW} + \Theta_{adi} \cdot SF_{adi} \tag{27}$$

$$Q = \Theta_Q \cdot SF_{LBW} \tag{28}$$

$$CL = \Theta_{CL} \cdot SF_{LBW}. \tag{29}$$

The scaling factors were defined in equation (23). The population parameters satisfy

$$\Theta_1 = \sum_{tis} \hat{K}_{tis,ref} \cdot V_{tis,ref} \tag{30}$$

where the sum is taken over all tissues of the central compartment;

$$\Theta_2 = \sum_{tis \# adi} \hat{K}_{tis,ref} \cdot V_{tis,ref} \tag{31}$$

where the sum is taken over all tissues of the peripheral compartment except adipose tissue; and

$$\Theta_{adi} = \hat{K}_{adi,ref} \cdot V_{adi,ref} \tag{32}$$

$$\Theta_Q = Q_{L,ref} \tag{33}$$

$$\Theta_{CL} = CL_{blood,ref} \tag{34}$$

All population parameters have a mechanistic interpretation. The volume of distribution at steady state $V_{ss,ref}$ is given by

$$V_{ss,ref} = \Theta_1 + \Theta_2 + \Theta_{adi}. \tag{35}$$

Alternative covariate models are

$$V_1 = \hat{\Theta}_1 \cdot LBW \tag{36}$$

$$V_1 = \hat{\Theta}_1 \cdot LBW + \hat{\Theta}_{adi} \cdot (BW - LBW) \tag{37}$$

$$Q = \hat{\Theta}_Q \cdot LBW \tag{38}$$

$$CL = \hat{\Theta}_{CL} \cdot LBW \tag{39}$$

where the population parameters $\hat{\Theta}_1$, $\hat{\Theta}_2$, $\hat{\Theta}_Q$, $\hat{\Theta}_{CL}$ are defined per kg LBW and $\hat{\Theta}_{adi}$ per kg adipose tissue. In this case, $V_{ss,ref} = (\hat{\Theta}_1 + \hat{\Theta}_2) \cdot LBW_{ref} + \hat{\Theta}_{adi} \cdot (BW_{ref} - LBW_{ref})$. It is important to notice that in this setting, it would not be meaningful to report a volume of distribution $\hat{\Theta}_1 + \hat{\Theta}_2 + \hat{\Theta}_{adi}$ per kg, as it would refer to both, kg LBW for $\hat{\Theta}_1$, $\hat{\Theta}_2$ and kg adipose tissue for $\hat{\Theta}_{adi}$.

METHODS

We used MATLAB R2010b (version 7.5; The MathWorks, Natick, MA) for modeling and simulation, and R (version 2.12.0) for statistical analysis.

Comparing different scaling approaches for predicting organ weights to experimental autopsy data. In the autopsy study by de la Grandmaison *et al.*,[14] organ weights of $n = 355$ male and $n = 329$ female Caucasians are reported. On the basis of the mean and SD of BH and BMI, we generated a virtual population of $n = 355$ male and $n = 329$ female individuals (**Supplementary Data** online for details), taking into account known correlations between BW and BH. The distribution of individual BH and BW is shown in **Figure 1** (top) for the male (left) and female (right) subpopulation. For each individual of the virtual de la Grandmaison population, we determined the organ weights using the LBW-scaling approach, and compared with the BH-scaling[9] and the regression equation approach.[10] For the experimental data of the autopsy study, a normal distribution was assumed as suggested in ref. 14 by reporting mean and SD. The conclusions, however, do not depend on this distributional assumption. No information on the correlation between different organ weights is provided.

Comparing the impact of different sources of interindividual variability on predicted blood concentration–time profiles. The impact of interindividual variability on anatomical and physiological parameters was assessed by predicting venous blood concentration–time profiles for the virtual de la Grandmaison population (subpopulation of 355 males only). To study the impact of variations in tissue partitioning, we used a virtual population of 355 males with identical BH = 1.72 and BW = 68 (mean values of virtual de la Grandmaison male subpopulation) and randomly perturbed partition coefficients. These perturbations were chosen to be uniformly distributed with twofold range of the original value (see **Supplementary Data** online, paragraph E.2) and thereby lower than the reported accuracy of the *in silico* prediction methods of a factor of 3.[29,41] We compared these two sources of variability based on the generic PBPK model, using lidocaine (400 mg via 60 min i.v. infusion) for illustrative purpose.

Illustrating the predictive power of the mechanistic covariate modeling approach. Illustrations are based on lidocaine (60 min i.v. infusion) with the two-compartment model being the minimal lumped model according to ref. 7.

Predicting volume of distribution and blood clearance in children: PBPK extrapolation vs. allometric scaling. On the basis of the PBPK model, we determined the age/sex dependent (blood) volume of distribution at steady state[7]:

$$V_{ss} = V_{art} + V_{ven} + \sum_{tis \neq liv} K_{tis} V_{tis} + (1 - E_{hep}) K_{liv} V_{liv} \qquad (40)$$

and the blood clearance

$$CL = \frac{Q_{liv} \cdot K_{liv} CL_{int}}{Q_{liv} + K_{liv} CL_{int}} \qquad (41)$$

For the allometric scaling approach, we used the adult values of V_{ss} and CL for male and female (as given by the PBPK model) to scale to children. To further quantify the differences, we determined the SD (σ) of

$$\frac{\text{Prediction}_{\text{Allometry}} - \text{Prediction}_{\text{PBPK}}}{\text{Prediction}_{\text{PBPK}}}$$

The resulting SDs for the 25 drugs with different physicochemical properties are listed in **Table 1**. As a reference, we further compared our PBPK extrapolation approach with PBPK extrapolations based on different parameter values for children, as proposed in literature (tissue volumes according to ref. 10 for V_{ss} or blood flows according to ref. 9 for CL). Willmann *et al.*[9] also uses the ICRP report for tissue volumes as we did, so we compared with Price *et al.* V_{ss} values. Regarding peripheral blood flows, the values obtained by Price *et al.* and Willmann *et al.* are almost identical, so we compared only with the latter approach. In both cases, all other parameters were chosen identical to our PBPK approach.

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Author contribution. All authors designed and performed the research, analyzed the data, and wrote the manuscript.

Conflict of interest. The authors declared no conflict of interest.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Covariate modeling is a key step in the analysis of clinical data. So far, no systematic approach exists to leverage the knowledge inherent in PBPK models in this context.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study addressed this problem by introducing (i) a novel approach to model inter-individual variability in PBPK models based on LBW; and (ii) a systematic approach to translate inter-individual variability into the design of mechanistic covariate models.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

The study established a novel approach to mechanistic covariate modeling and as a further result a new covariate relation for the volume of distribution at steady state that seamlessly integrates body weight and LBW as covariates.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS

The study mechanistically justified %LBW as an important characteristic when aiming at studying drug PK in obese individuals. It further suggested indicators that one may look at preclinically to predict outcomes in the clinic (e.g., K_{adi} to K_{tis} ratio).

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Supplementary Information accompanies this paper on the *CPT: Pharmacometrics & Systems Pharmacology* website (http://www.nature.com/psp)