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Population Pharmacokinetics of Atorvastatin and Its Active Metabolites in Children and Adolescents With Heterozygous Familial Hypercholesterolemia: Selective Use of Informative Prior Distributions from Adults

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Abstract

The population pharmacokinetics (PPK) of atorvastatin and its principal active metabolite, *o*-hydroxyatorvastatin, were described in 6–17 years old pediatric hypercholesterolemia patients with a 2-compartment model for both parent and metabolite. Informative prior distributions on selected parameters, based on adult data, were required to stabilize the model and were implemented using a Bayesian penalty term on the likelihood function in the nonlinear mixed effects model (NONMEM VI with PRIOR). Concentrations below the limit of quantitation were treated as censored data using a conditional likelihood function. Atorvastatin apparent oral clearance (CL/F) was described as a function of body weight using an allometric equation. Based on the final model, the typical CL/F estimates for a Tanner Stage 1 patient (35 kg weight) and Tanner Stage $\geq$2 (50 kg weight), would be 553 and 543 L/hour, respectively. When scaled allometrically, CL/F was similar to values reported for adults. Variability in atorvastatin PK was primarily affected by weight.

Keywords

atorvastatin, pediatric, hypercholesterolemia, population pharmacokinetic, metabolites, informative prior distributions, Bayesian

Atorvastatin (ATV) is a lipid-lowering agent, approved for treatment once daily at 10–80 mg doses in adults and at 10–20 mg doses in children aged 10 years or older.[1] ATV is orally administered in the open hydroxy acid ring form. In humans, atorvastatin is extensively metabolized to active metabolites, *ortho*- and *para*-hydroxyatorvastatin. ATV and both its active metabolites undergo lactonization, and are in equilibrium with their respective inactive lactone forms *in vivo*.[2] *O*-hydroxyatorvastatin (*o*-ATV) is the predominant active metabolite in systemic circulation.[3,4] *In vitro* inhibition of HMG-CoA reductase by *ortho*- and *para*-hydroxylated metabolites is equivalent to that of ATV. Approximately 70% of circulating inhibitory activity for HMG-CoA reductase is attributed to active metabolites.[1]

Although ATV is indicated as lipid lowering therapy in children at 10 years or older, the pharmacokinetics (PK) of ATV has not been characterized in pediatric patients. In addition, the development of an appropriate pediatric formulation had been identified as a unmet pediatric need in cardiovascular products by the Pediatric Working Party, a view also adopted and published by the CHMP.[5] A chewable tablet formulation was developed as an alternative treatment option, together with availability of a lower-strength dose (5 mg) for use with younger pediatric patients, as part of a Pediatric Investigation Plan recommended in the Written Request by the EMA Paediatric Committee (PDCO).[6] A study to characterize the pharmacokinetics of atorvastatin and its metabolites in pediatric patients with heterozygous familial hypercholesterolaemia (HeFH) using 10 mg daily doses of a marketed tablet formulation for older children and 5 mg daily doses of a chewable tablet formulation for younger children was prospectively designed in consultation with the investigators and the European Medicine Agency (EMA).[6] Recruitment in this trial was stratified based on Tanner Stage (TS), a stage of sexual maturity rating.[7,8]

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Typically younger children of age 6–10 years are at TS 1; older children of age 10–17 years are at TS ≥2.

Pediatric trials are limited by the number of PK samples that can be collected (both the number of patients and the number of samples/patient) and are therefore not amenable to traditional methods of pharmacokinetic analyses. Specifically for pediatric pharmacokinetics analysis of parent and its metabolites, population PK methods are particularly useful when combined with the selective use of prior information from adult studies.[9] The objective of this pediatric population PK analysis is to provide a detailed description of pharmacokinetics for ATV and its active metabolites (*o*-ATV and *p*-hydroxyatorvastatin (*p*-ATV)) in children with HeFH, and to examine the influence of covariates on the PK parameters.

Methods

Study Design

The pediatric study was an 8-week, open-label trial that enrolled 15 TS 1 and 24 TS ≥2 genetically verified HeFH children with low-density lipoprotein cholesterol (LDL-C) >4 mmol/L at baseline. The initial dose for TS ≥2 children was atorvastatin 10 mg daily of a marketed tablet formulation; the initial dose for TS 1 children was atorvastatin 5 mg daily of a chewable tablet formulation, bioequivalent to the marketed tablet. The doses were doubled at Week 4 if a subject had not attained target LDL-C of <3.35 mmol/L. Other patient population and study design details have been reported elsewhere.[10] A total of 8 sparse blood PK samples (2 mL each) were obtained per subject: one blood sample (between 4 and 12 hours postdose) each was collected at Weeks 2 and 6, and 3 blood samples (at predose and at 1 and 2 hours postdose during each clinic visit) each were collected at Weeks 4 and 8. Taking into account the sample size of the pediatric study and the adult PPK models, the sampling scheme was optimized for precise model parameter determination, with the consideration to minimize blood sampling and inconvenience in pediatric subjects. Plasma samples were analyzed for ATV, *o*-ATV, and *p*-ATV using a validated liquid chromatography (LC)–mass spectrometry (MS)/MS analytical method at Advion Bioservices, Ithaca, NY.[11] The assays demonstrated a linear range of 0.250–100 ng/mL, with the lower limit of quantification (LLOQ) of 0.250 ng/mL. The concentrations of the quality control (QC) samples were 0.750, 2.00, 8.00, 80.0, and 240 ng/mL. During sample analysis, the accuracy (expressed as the percent difference from the theoretical concentration) of the QC samples ranged from −6.0% to 0.9%, −1.0% to 3.9%, and −4.5% to 3.4% for ATV, *o*-ATV, and *p*-ATV, respectively. The precision (expressed as the percent coefficient of variation) during sample analysis was ≤6.9%, ≤13.5%, and ≤5.7% for ATV, *o*-ATV, and *p*-ATV, respectively.

Data Assembly and Data Analysis

The data sets from this pediatric study and 3 adult studies were utilized for this analysis.[12–15] ATV dose and ATV, *o*-ATV, and *p*-ATV concentrations were converted to their molar equivalents, where the molecular weights of ATV, *o*-ATV, and *p*-ATV are 558.64, 574.64, and 574.64, respectively.

Population PK analyses were conducted via nonlinear mixed effects modeling with the NONMEM® software, Version VI, Level 2.0 (ICON Development Solutions, Ellicott City, MD). The first-order conditional estimation with interaction (FOCEI) in NONMEM was employed for all model runs.[16] For those PK models implementing the truncated likelihood for BQL concentrations, the first-order conditional estimation with interaction laplacian (FOCEIL) was used.[17]

Model selection was driven by the data and was based on various goodness-of-fit indicators, including comparisons based on the minimum objective function value (OFV), visual inspection of diagnostic scatter plots, and evaluation of estimates of population fixed- and random-effect parameters.[16]

The impact of individual-specific covariates was assessed as part of the PK model development. Specific effects that were estimated include the effects of age and weight as predictors of ATV apparent oral clearance (CL/F), the effect of weight as a predictor of apparent central and peripheral volumes of distribution (Vc/F and Vp/F) and apparent inter-compartmental clearance (Q/F), and the effect of Tanner Stage on relative bioavailability (F1). A full covariate model approach, based on estimation of pre-specified covariate effects, was employed for these analyses.[18] This approach allows for the assessment of clinical relevance of covariate effects without the need to assess statistical significance.

Analysis Methods for Pediatric Data

Strategies for the model-based analysis of pediatric population PK data are dependent upon the information content in the available pediatric data set. Often times the pediatric data are limited due to restrictions on blood sampling and/or the willingness of pediatric patients to participate in PK and PK/PD studies. In the limited data situation, the PK analysis strategies can typically be described by four general approaches:

- The first method consists of analyzing the pediatric data alone, intentionally utilizing a mis-specified, but simpler structural model that may be supported under the limited data set, for example, the use of a 1-compartment disposition rather than a 2-compartment model (Simple Model).
- The second method involves analysis of the pediatric data alone under the known structural

model, by fixing the PK parameters that are poorly supported under the current data set to some "known" value based on prior information (most likely based on adult studies or expert opinion; Fixed Model).
- The third method utilizes the known model structure, but avoids the necessity of fixing model parameters, by pooling sparsely sampled pediatric PK data sets with more informatively designed adult PK data sets in a single analysis (Pooled Model).
- The fourth method applies Bayesian estimation methods or concepts by estimating the parameters of the known structural model, using the pediatric data alone, with parameters supported by the selective use of informative prior distributions obtained from adult studies[9] (BayesPrior). The application and results of these strategies in the current analysis are described in the Results Section.

As a starting point for this analysis, all inter-individual error terms were described by an exponential error model or log-normal parameter distribution (Eq. 1). An attempt was made to define a full block covariance matrix for the inter-individual random effects (Ω) when possible

$$P_i = \hat{P} * \exp(\eta_{Pi}) \quad (1)$$

where P_i is the estimated parameter value for individual i, $\hat{P}$ is the typical population value (geometric mean) of the parameter, and η_{pi} are individual-specific inter-individual random effects for individual i and parameter P and are assumed to be distributed: $\eta \sim N(0, \omega^2)$ with covariances defined by the inter-individual covariance matrix.

For pharmacokinetic observations in this analysis, the residual error model was initially described by a combined additive and proportional error model (Eq. 2)

$$C_{ij} = \hat{C}_{ij}(1 + \varepsilon_{pij}) + \varepsilon_{aij} \quad (2)$$

where C_{ij} is the jth measured observation in individual i, $\hat{C}_{ij}$ is the jth model predicted value in individual i, and ε_{pij} and ε_{aij} are proportional and additive residual random errors, respectively, for individual i and measurement j and are each assumed to be independently and identically distributed: $\varepsilon \sim NID(0, \sigma^2)$.

For pharmacokinetic models incorporating BQL observations, an adjusted likelihood function was implemented, treating the BQL data points as censored observations. This approach is described as the M3 method by Beal[19] and Ahn et al[17] Briefly, the M3 method allows BQL observations to be retained in the dataset but handles them as censored observations. For models implementing the M3 method, a proportional residual error model and the FOCEIL were used.

For pharmacokinetic models incorporating prior adult information without the use of the previous adult concentration–time data, the NONMEM PRIOR subroutine was implemented. This approach has been described by Gisleskog et al,[20] Gastonguay et al,[21] and in the NONMEM Users Guide.[16] The PRIOR subroutine allows a penalty function based on a frequency prior to be specified and added to the −2 log likelihood objective function.[16] Adult prior information to support estimation of fixed effects and covariance matrix of the inter-individual random effects enter the model via the specification of hyperparameters describing the prior distributions on these population-level parameters. These hyperparameters are described using a multi-variate normal (MVN) distribution for fixed-effect parameters (e.g., THETA vector) and an inverse-Wishart distribution ($Wishart^{-1}$) for the covariance matrix of inter-individual random effects (e.g., OMEGA matrix; Eq. 3)

$$\begin{aligned} \Theta_{prior} &\sim N(\mu, \Sigma) \\ \Omega_{prior} &\sim Wishart^{-1}(\Psi, m) \end{aligned} \quad (3)$$

where Θ_{prior} is the described by a normal distribution, N, with mean μ and covariance matrix Σ, Ω_{prior} is described by an inverse-Wishart distribution, $Wishart^{-1}$, with scale matrix Ψ and degrees of freedom m.

The use of the hyperparameters serves as a prior constraint on these population-level parameters and allows stable estimates of these parameters to be obtained in situations when the available data is insufficient. The relative influence of the informative prior distributions can be modulated by varying the magnitude of the prior multivariate normal covariance matrix for THETA (where an increase in the variance modulates the prior from one that is more informative to one that is more vague), and by varying the magnitude of the degrees of freedom in the inverse-Wishart prior for OMEGA (where an increase in degrees of freedom modulates the prior from one that is more vague to one that is more informative).

A fixed allometric model was used to incorporate the effect of weight on the PK parameters. The use of a fixed allometric model is the standard approach for analyzing pharmacokinetics in the pediatric population. It has been shown that the change in physiologic parameters as a function of body size is both theoretically and empirically described by an allometric model.[22–25]

Model Evaluation

The final model and parameter estimates were investigated with a predictive check method. This method is similar to the previously described posterior predictive check, but assumes that parameter uncertainty is negligible relative to inter-individual and residual variance.[26,27] Five hundred

Monte Carlo simulation replicates of the original data set were generated using the final population PK model. Distributions of the median observed concentration (CMED) across all data points within each individual from the simulated data were compared with the distribution of the CMED in the observed data set, using exploratory graphics (quantile–quantile plots).

The precision of model parameters was investigated by performing a stratified non-parametric bootstrap procedure.[28–31] One thousand replicate data sets were generated by random sampling with replacement and were stratified by Tanner Stage, using the individual as the sampling unit. Population parameters for each data set were subsequently estimated using NONMEM. Empirical 95% confidence intervals (CI) were constructed by observing the 2.5th and 97.5th quantiles of the resulting parameter distributions for all bootstrap runs.

Results

Analysis Population and Data Characteristics

The pediatric PK data were comprised of 39 patients contributing a total of 310 plasma samples. Patient demographics at screening are summarized in Supplemental Table S1. There were 15 Tanner Stage 1 and 24 Tanner Stage $\geq$ 2 patients. Age and weight ranged from 6 to 17 years and 25–99.4 kg, respectively. Age and weight distributions across Tanner Stages were consistent with the underlying criteria used for the category, mainly, age and weight are lower in Tanner Stage 1 when compared to Tanner Stage $\geq$ 2 patients (data not shown).

The population PK dataset contained 51 (16% of total) and 37 (12% of total) concentrations that were BQL for ATV and *o*-ATV, respectively. The plasma concentrations of *p*-ATV were BQL in a majority (>80%) of the plasma samples collected in this study. The degree of BQL data across three analytes is consistent with the findings in a recent adult bioequivalence study at a 10 mg dose of atorvastatin (Pfizer internal data).[12] Therefore, the population PK modeling was pursued with a model for ATV and its measurable active metabolite, *o*-ATV.

Population Pharmacokinetic Modeling Results—Atorvastatin

As suggested by the prior population analysis in adults,[32] a 2-compartment model with first-order absorption was chosen as the base structural model. The model was parameterized in terms of apparent ATV clearance (CL/F), apparent ATV central volume of distribution (Vc/F), apparent ATV intercompartmental clearance (Q/F), apparent ATV peripheral volume of distribution (Vp/F), and absorption rate constant (Ka; Supplemental Eq. S1). The L2 was calculated and incorporated into the calculation of Ka to avoid "flip-flop" kinetics. Inter-individual random effect distributions were modeled using exponential variance models, with a covariance term between CL/F and Vc/F, while residual random effects were described with a combined additive and proportional model. In addition, CL/F, Vc/F, Q/F, and Vp/F were allometrically scaled by weight using a power model with the exponents fixed to 0.75 for CL/F and Q/F and 1 for Vc/F and Vp/F.

Attempts to utilize a simpler model or to fix certain PK parameters (Simple Model and Fixed Model methods) yielded less than optimal results. The models with a fixed Ka resulted in unrealistically large estimates for Vp/F. When Ka and Vp/F were fixed, the Vc/F estimate was unrealistically small (Supplemental Table S2). Attempts to simplify the PK structural model to 1-compartment rather than 2-compartment also yielded unrealistically large Vc/F and Ka point estimates.

Pooling of the pediatric data with adult data to anchor the portions of the model that were not well described by the pediatric data alone did not provide the stability that was expected. Initially, the single-dose adult data[31–33] were used to build a population PK model for atorvastatin in adults only, using the 2-compartment model described in Supplemental Equation (S1). These results demonstrated that the PK parameter point estimate across all of the adult studies were quite variable (Supplemental Table S3). In addition, potential confounding existed between PK parameter differences and study design (e.g., single-dose, rich PK sampling vs. multiple-dose sparse sampling). Given this variability in parameter estimates and study design conditions, it was unclear which studies would be most appropriately pooled with the sparsely sampled pediatric data set. There was also a concern that the large number of subjects and samples in the adult studies would overwhelm the small pediatric trial data set.[9] Instead, modeling approaches that borrowed prior information from adults, but limited the influence of the adult data were utilized.

An approach that formally includes prior information from adults to selectively inform a subset of pediatric population PK model parameters was implemented. This approach utilized the NONMEM PRIOR subroutine as described in the Methods section and prior information derived from the previous population PK analysis in adults[29] (Supplemental Table S4). The results from this analysis were used to derive the priors because it provided PK information in patients over a range of doses. In this implementation, the PK model fixed-effect parameters were log-transformed to keep the PK model consistent with the MVN assumption for the THETA prior and to provide some additional stability during the estimation process.

The priors (presented on a log scale) developed from the adult atorvastatin population PK analysis are shown in Equation (4). The mode for the MVN distribution for the THETA priors was described by the final fixed-effect

parameter estimates and are shown going from top to bottom in Equation (4) for log(CL/F), log(Vc/F), log(Q/F), log(Ka), and log(Vp/F). The variance for the THETA priors was described as the squared standard error for each parameter estimate. The Wishart^{-1} prior for the inter-individual random effects was described using the var–cov matrix of the inter-individual random effects estimated from the adult model. As shown below, the CL/F, Vc/F, and Q/F priors were vague, with a variance of 1×10^6.

$$\begin{aligned}\Theta_{\text{prior}} &: \text{N}\left(\begin{pmatrix}5.65\\5.60\\4.29\\-1.33\\7.07\end{pmatrix}, \begin{bmatrix}10^6 & 0 & 0 & 0 & 0\\0 & 10^6 & 0 & 0 & 0\\0 & 0 & 10^6 & 0 & 0\\0 & 0 & 0 & 0.00684 & 0\\0 & 0 & 0 & 0 & 0.0156\end{bmatrix}\right)\\ \Omega_{\text{prior}} &: \text{Wishart}^{-1}\left(\begin{bmatrix}0.1003 & 0.06034\\0.06034 & 0.1003\end{bmatrix}, 4\right)\end{aligned} \tag{4}$$

During the model building process, the informativeness of the adult priors, as specified by the hyperparameters for the fixed effects, was varied to arrive at a pediatric model that minimized the influence of adult prior information but still allowed for the stable estimation of the fixed-effect parameters. The CL/F prior was intentionally vague for all of the models evaluated to allow the pediatric data alone to drive the estimation of this parameter. The Wishart^{-1} distribution utilized to describe the prior for the OMEGA matrix was varied in some of the models but was ultimately fixed to the smallest possible degrees of freedom to ensure the estimation of the inter-individual random effects was driven by the pediatric data rather than the prior adult information.

Initial model building indicated an informative prior was required on Ka and Vp/F to stabilize the model. This resulted in plausible and precise fixed-effect (% SE $< 10\%$) parameters, while random-effect parameters were estimated with less precision (Table 1, base model). All subsequent models maintained the informative prior on Ka and Vp/F but allowed the pediatric data to drive the remaining parameters.

Due to the percentage of BQL observations in the data set, the M3 method for handling BQL's was implemented.[17,19] From this point on in the modeling process, the molar equivalents for atorvastatin dose and concentration were utilized and BQL concentrations were added into the NONMEM dataset. The fixed and inter-individual random effects portion of the model were identical to that described in Supplemental Equation (S1) and the random effects portion of the model was altered to account for the

Table 1. Pharmacokinetic Parameter Estimates from ATV Base Model, Base Model With BQL, and Full Model With BQL PK Analysis (Using 70 kg as Reference Weight)

	Point Estimate (% SE)			
	Base Model	Base Model With BQL	Final Model With BQL	Prior Type
CL/F (L/h) $= \theta_1$	807 (1.32%)	787 (1.3%)	652 (1.71%)	Vague
CL/F $\sim$ (WT/70)$^{0.75}$	—	—	—	
Vc/F (L) $= \theta_2$	1610 (3.53%)	1570 (3.72%)	1140 (3.95%)	Vague
Vc/F $\sim$ (WT/70)^{1}	—	—	—	
Q/F (L/h) $= \theta_3$	361 (4.84%)	336 (5.17%)	326 (5.1%)	Vague
Q/F $\sim$ (WT/70)$^{0.75}$	—	—	—	
Vp/F (L) $= \theta_5$	2030 (1.46%)	2030 (1.48%)	1920 (1.45%)	Informative
Vp/F $\sim$ (WT/70)^{1}	—	—	—	
Ka (h^{-1}) $= \theta_4$	0.238 (8.36%)	0.239 (8.58%)	0.235 (7.59%)	Informative
F1-Tanner Stage 1 $= \theta_6$	—	—	0.581 (7.59%)	None
F1-Tanner Stage 2 $= \theta_7$	—	—	1 (NA)	None
$\Omega^{1,1}$CL/F	0.269 (23.2%) CV = 51.8%	0.249 (24.2%) CV = 49.9%	0.313 (25.3%) CV = 56%	Vague
$\Omega^{1,2}$COV$_{\text{CL/F–Vc/F}}$	0.170 (51.6%) r = 0.347	0.166 (49.9%) r = 0.374	0.157 (59.3%) r = 0.337	Vague
$\Omega^{2,2}$Vc/F	0.895 (27.2%) CV = 94.6%	0.797 (26.8%) CV = 89.3%	0.694(27.3%) CV = 83.3%	Vague
$\sigma^{1,1}$pro	0.150 (11%) CV = 38.8%	0.169 (5.56%) CV = 41.1%	0.166 (5.46%) CV = 40.6%	None

ATV, atorvastatin; CL/F, ATV clearance; Vc/F, ATV volume of distribution; Q/F, ATV inter-compartmental clearance; Vp/F, ATV peripheral volume of distribution; Ka, first-order absorption rate constant; % SE, percent standard error; CV, coefficient of variation; Ω, inter-individual variance or covariance (COV); σ, residual variance; r, correlation; NA, not applicable.

inclusion of BQL observations.[17] The results of the model incorporating the ATV BQL observations were almost identical to those from the model without BQL observations (Table 1). However, the M3 method for BQL handling was kept in the model due to the relatively large percentage of BQL values in the data set.

The ATV base structural model provided an adequate description of the data, as judged by visual inspection of diagnostic plots (Supplemental Figure S1). The base model structural parameter estimates, presented in Table 1 (base model with BQL), were estimated with good precision. Variance parameter estimates were indicative of a relatively large degree of unexplained inter-individual variability in CL/F (49.9% CV) and Vc/F (89.3% CV). Distributions of inter-individual random effects were centered at the expected value of zero, as indicated by the ETA-BAR estimates included in the NONMEM output (data not shown). Shrinkage estimates for the η's were small indicating trends in exploratory graphics are not being masked by η-shrinkage. The negative CL/F η-shrinkage value is likely indicative of the difficulty in estimating the standard deviation of a normal distribution using 39 patients rather than a problem with the η estimate (Supplemental Table S5).

The primary covariates of interest had been pre-defined for this analysis and included age and weight as predictors of CL/F, weight as a predictor of Vc/F, Vp/F, and Q/F, and the effect of Tanner Stage on F1. Weight was present in the base model as a fixed allometric relationship so Tanner Stage was added to the base model as a predictor of F1 to create a full covariate model. Given that the Tanner Stage classification incorporates effects of formulation, age and dose, it was selected as a practical covariate to represent all of these effects and was incorporated as an effect on F1.

The ATV full model resulted in similar goodness-of-fit criteria, compared to the base model (Table 1, full model and Figure 1, top panel). No trends were evident in plots evaluating the effect of covariates on random effects (data not shown). The η-shrinkage estimates for this run were small and similar to those from the base model and also demonstrated a negative shrinkage for the η on CL/F (Supplemental Table S5). The full model for atorvastatin was carried forward as the ATV portion of the combined ATV/*o*-ATV parent-metabolite model.

Population Pharmacokinetic Modeling Results—Combined Parent and Metabolite Model

A 2-compartment metabolite model, as suggested by graphical inspection of the *o*-ATV concentration–time data and the similar ATV and *o*-ATV molecular weight, structure, and log P values, was added to the existing 2-compartment parent model. The metabolite model was parameterized in terms of *o*-ATV apparent oral clearance (CLm/fm), apparent oral central volume (Vcm/fm), apparent inter-compartmental clearance (Qm/fm), apparent peripheral volume of distribution (Vpm/fm), and fraction metabolized (fm). The combined parent-metabolite model is shown in Supplemental Equation (S2) and a diagram of the model can be found in Figure 2. For the metabolite portion of the model, an inter-individual random effect distribution was modeled on CLm/fm using an exponential variance model, while the residual random effect for *o*-ATV was described with a proportional model. The M3 method for accounting for BQL values was utilized for the *o*-ATV random residual model. In addition, CLm/fm, Vcm/fm, Qm/fm, and Vpm/fm were allometrically scaled by weight using a power model with fixed exponents.

Under the current pediatric study experimental design, the exact values of the metabolite portion of the model, described in Supplemental Equation (S2), are mathematically unidentifiable. For mathematical identifiability, either a complete mass balance of metabolite sampling, or a separate experiment including exogenous administration of *o*-ATV are required. Another method to create an identifiable set of model parameters is to fix either fm or Vcm/fm. For this analysis, the results of a number of interim models indicated fixing the value of fm to 1 was the best approach to ensure mathematical identifiability. This resulted in the estimation of apparent metabolite PK parameters that are proportional to the "true" values, adjusted by the fraction metabolized as proportionality constant.

The parent-metabolite modeling was performed with a goal of determining a combined model that provides a good description of ATV and *o*-ATV concentrations while resulting in a set of ATV model parameters similar to those determined from a fit of the ATV only data (Table 1). A variety of models were tested but the model providing the best fit utilized informative priors on Vcm/fm, Vpm/Ffm, and Qm/Fm and fm fixed to 1 (Supplemental Table S6, Run 340). The informative priors for the metabolite portion of the combined model were derived from the results of the ATV full model (Table 1) and are displayed in Equation (5). The mode for the MVN distribution for the metabolite THETA priors was described by the final fixed-effect parameter estimates of Vc/F, Q/F, and Vp/F from Table 1 (full model with BQL) and are shown from top to bottom in Equation (5) for log(Vcm/fm), log(Qm/fm), and log(Vpm/fm). The variance for the THETA priors was described as the squared standard error from these parameters (Vc/F, Q/F, and Vp/F). There were no priors placed on the CLm/fm and inter-individual random-effect portions of the metabolite model so the estimates of these parameters would be primarily driven by the available pediatric data. The priors for the parent portion of the combined model were the same as those used in the ATV only population PK

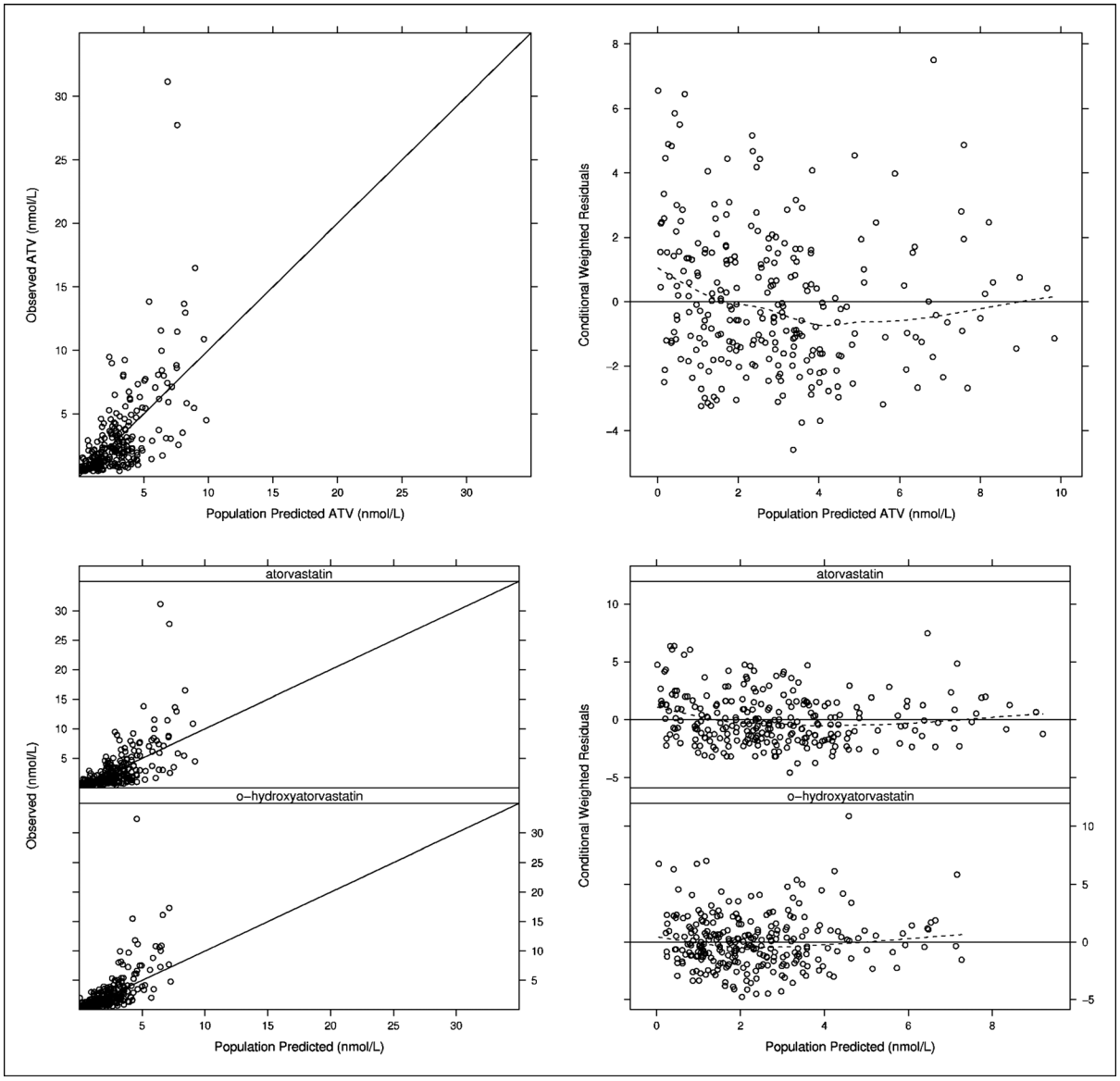

Figure 1. Diagnostic plots for ATV models. Observed concentrations are plotted versus population predictions (left) and CWRES are plotted versus population predictions (right). Values are indicated by open circles. The line of identity (solid black) is included as a reference (left) or a dotted black lowess trend line is included (right). Final ATV only model in top panel and final parent-metabolite model is in lower panel with ATV on top and *o*-ATV on bottom in lower panel.

model (Eq. 4)

$$\Theta_{\text{metabolite\_prior}} : \text{N}\left(\begin{pmatrix}7.02\\5.78\\7.56\end{pmatrix}, \begin{bmatrix}0.0778 & 0 & 0\\0 & 0.0870 & 0\\0 & 0 & 0.0119\end{bmatrix}\right) \quad (5)$$

The combined parent-metabolite model provided a good description of the ATV and *o*-ATV data, as judged by visual inspection of diagnostic plots (Figure 1, bottom panel). Distributions of inter-individual random effects were centered at the expected value of zero, as indicated by the ETA-BAR estimates included in the NONMEM output. η-shrinkage estimates for the final model were small indicating that covariate trends are not likely to be masked (Supplemental Table S7).

Parameter estimates from the final parent-metabolite model are presented in Table 2. For Tanner Stage 1 patients, the typical estimate (95% CI) for F1 was 0.752 (0.577, 1.01). There was a relatively large degree of inter-individual variability in CL/F (46.3% CV), Vc/F (106% CV), and CLm/fm (43.3% CV). Along with the parameter point estimates, measures of parameter estimation uncertainty (95% CI), determined by non-parametric

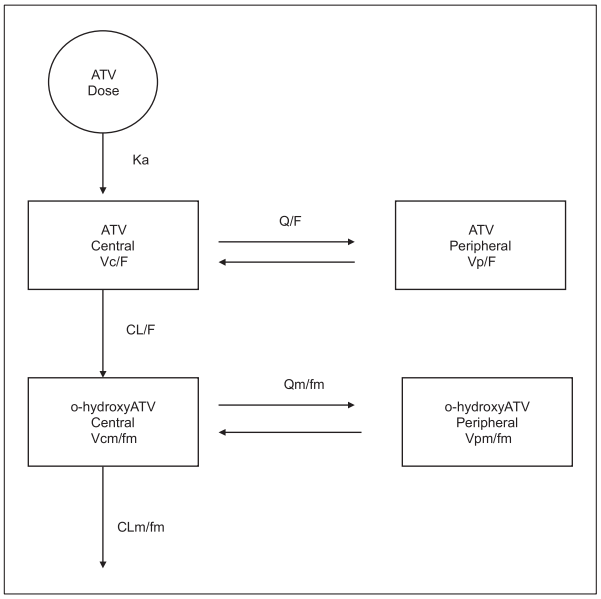

Figure 2. Diagram of atorvastatin parent-metabolite model.

Table 2. Pharmacokinetic Parameter Estimates from Final Parent-Metabolite Population Pharmacokinetic Model (Run 340; Using 70 kg as Reference Weight)

	Point Estimate	Bootstrap CI	Prior Type
CL/F (θ_1)	699 (L/h)	(570, 881)	V
CL/F $\sim$ (WT/70)$^{0.75}$			
Vc/F (θ_2)	1020 (L)	(209, 2210)	V
Vc/F $\sim$ (WT/70)^{1}			
Q/F (θ_3)	277 (L/h)	(80.2, 470)	V
Q/F $\sim$ (WT/70)$^{0.75}$			
Vp/F (θ_5)	1960 (L)	(1390, 2460)	I
Vp/F $\sim$ (WT/70)^{1}			
Ka (θ_4)	0.2 (h^{-1})	(0.139, 0.304)	I
CLm/fm (θ_{10})	616 (L/h)	(518, 748)	N
CLm/fm $\sim$ (WT/70)$^{0.75}$			
Vcm/fm (θ_6)	401 (L)	(272, 715)	I
Vcm/fm $\sim$ (WT/70)^{1}			
Qm/fm (θ_7)	368 (L/h)	(248, 562)	I
Qm/fm $\sim$ (WT/70)$^{0.75}$			
Vpm/fm (θ_8)	2040 (L)	(1740, 2250)	I
Vpm/fm $\sim$ (WT/70)^{1}			
F1-Tanner Stage 1 (θ_{12})	0.752	(0.577, 1.01)	N
F1-Tanner Stage 2 (θ_{11})	1	NA	N
$\Omega^{1.1}$CL/F	0.124 (CV = 46.3%)	(0.125, 0.293)	V
$\Omega^{1.2}$COV$_{CL/F-Vc/F}$	0.185	(−0.139, 0.35)	V
$\Omega^{2.2}$Vc/F	1.12 (CV = 106%)	(0.721, 3.64)	V
Ω^{33}CLm/fm	0.188 (CV = 43.3%)	(0.0868, 0.288)	N
$\sigma^{1.1}$pro-ATV	0.17	(0.124, 0.207)	N
$\sigma^{2.2}$pro-*o*-ATV	0.166	(0.177, 0.207)	N
Residual variability as CV			
ATV CV	41.2 (CV%)	(35.2, 45.5)	N
orthoATV CV	40.6 (CV%)	(34.2, 45.5)	N

ATV; atorvastatin; CL/F; ATV clearance; Vc/F; ATV volume of distribution; Q/F; ATV inter-compartmental clearance; Vp/F; ATV peripheral volume of distribution; Ka; first-order absorption rate constant; fm; fraction of bioavailablc does converted to metabolitc; CLm/fm; *o*-ATV CL; Vcm/fm; 0-ATV volume of distribution; Qm/fm; *o*-ATV intercompartmental clearance; Vpm/fm; *o*-ATV peripheral volume of distribution; F1; relative bioavailability; CI; confidence interval, Ω; inter-individual variance or covariance (COV); σ; residual variance; CV; coefficient of variation; V; vague; I; informative; N; none; NA; not applicable.

bootstrap, were also obtained. Typical structural model parameters, with the exception of Vc/F, and random variance parameters were estimated with good precision.

The weight effects on CL/F, Vc/F, Q/F, Vp/F, CLm/fm, Qm/fm, Vcm/fm, and Vpm/fm were fixed allometric relationships. The covariate effect plots describing this relationship demonstrate the proportional change, relative to the reference 70 kg individual, and precision in the PK parameter at weights of 25, 35, 50, and 65 kg. Comparing the extremes of this range, the typical value of CL/F would be expected to be 50% lower for a 25 kg patient relative to a 70 kg patient. This difference reflects the fixed allometric relationship used to describe the effect of weight in the pediatric patients (Figure 3, left).

The effect of Tanner Stage 1 on F1 demonstrated a 95% CI that ranged from 0.57 to the null value of 1, with the probability density for this effect centered at a value that is approximately 25% less than the reference value. Results demonstrated a 54% probability that the estimated effect is within 25% of the reference value, with a 46% probability that the effect is more than 25% lower than the reference Tanner Stage $\geq$ 2 effect (Figure 3, right).

Model Evaluation

The ATV and *o*-ATV combined population PK model evaluation results, which included the results of a predictive check and a non-parametric bootstrap, revealed that the final model provided a reliable description of the data with good precision of most structural model and variance parameter estimates.

The predictive performance of the final population PK model was assessed using the median observed concentration CMED within each individual as the data characteristic of interest. For each of 500 simulation replicates of the original data set, the entire distributions of simulated CMED values were compared to the observed distribution using quantile–quantile plots (Q–Q plots). Simulated distributions of ATV and *o*-ATV were in good agreement with the observed data for pediatric subjects (Figure 4).

Discussion

Strategies for the model-based analysis of pediatric population PK data depend upon the information content in the available pediatric data set. When a sparse sampling

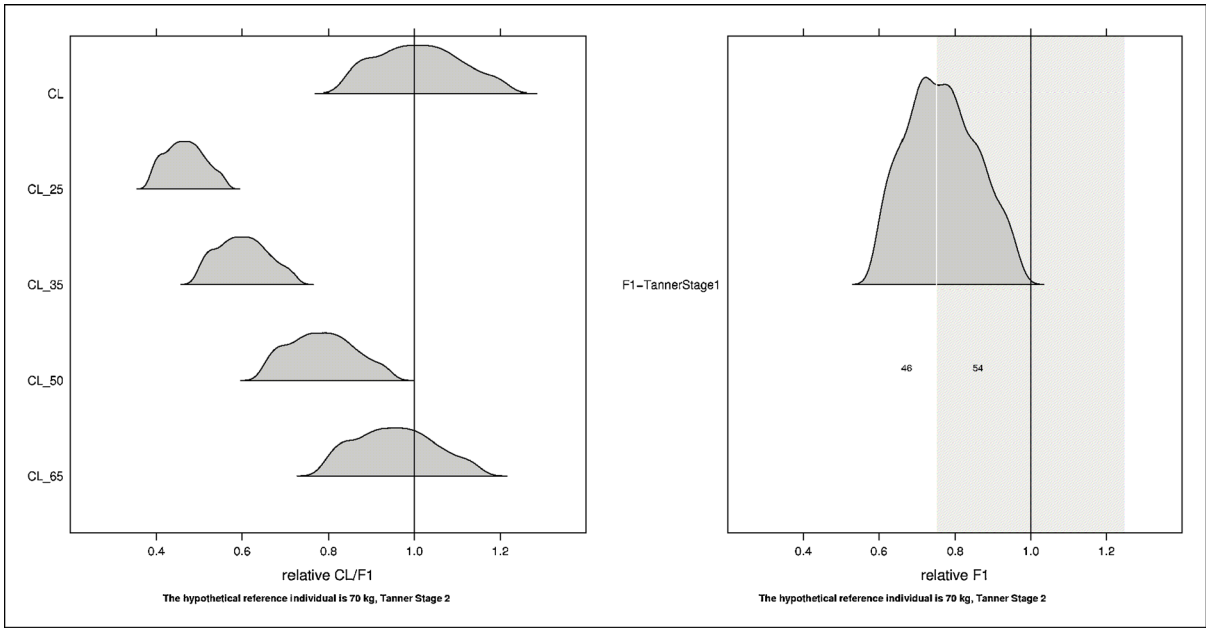

Figure 3. Covariate effect of weight on CL/F (left) and Tanner Stage on F1 (right). The horizontal lines represent the 95th percentiles of nonparametric bootstrap estimates for the named PK parameter, and so represent the relative precision for that PK parameter (CL/F). The distributions (95th percentiles) of the 999 nonparametric bootstrap estimates are provided as density smooths atop each horizontal range. All evaluations are normalized by dividing by the named PK parameter's point estimate (from Final Model)—thus, a value of one (1) represents unity or a null covariate effect. For continuous covariates (e.g., weight), the estimates are provided at lower, intermediary, and upper values of the observed covariate's range for each parameter (e.g., CL_25, CL_35, CL_50, or CL_65) corresponding to the parameter's relative (proportional) value for a patient weighing 25, 35, 50, or 65 kg, respectively). For Tanner Stage, the reference value is 1 and the plot represents the distribution of F1 estimates from the 999 non-parametric bootstrap runs. The gray shaded area represents the null value ±25% and is intended to reflect an area in which the covariate effects represented a minimal change from the null effect. The numbers on the plot for each effect estimate, from left to right, represent the % of the 999 nonparametric bootstrap estimate that were below −25%, between −25% and the null, between the null and 25%, and above 25% of the null.

pediatric trial design has been optimized for accurate and precise estimation of population fixed- and random-effect parameters under a proposed structural model, it is sufficient to analyze the pediatric data alone under the proposed model. In many situations, however, it is not practical to implement an optimal pediatric PK sampling design. Data are often limited (e.g., number of patients and number of observations/patient) due to restrictions on the volume of blood sampled, willingness of pediatric patients to participate in PK studies, and the practical timing of PK sampling given other interventions and activities. The resulting data set may, therefore, provide insufficient information to support estimation of all parameters of the proposed model.

To address the limited data in the pediatric ATV study, four general methods were proposed. The application of the: SimpleModel, FixedModel, and PooledModel methods did not yield reliable parameter estimates. The BayesPrior method with the selected use of informative priors enabled the simultaneous description of the ATV and *o*-ATV PK data via the application of a combined parent-metabolite model. Combined parent-metabolite models are more clinically relevant because they allow simultaneous description of the ATV and *o*-ATV concentrations and the relationship of parameters within individuals including covariate effects on PK.

Early on in the parent-metabolite modeling process it became apparent that informative priors would be necessary on the Ka and Vp/F parameters of the parent portion and on the Vcm/fm, Qm/fm, and Vpm/fm parameters of the metabolite portion of the combined model. Models that did not utilize priors and a model that utilized a prior only on Ka resulted in unreliable estimates of Ka and/or Vp/F. The informative priors for the metabolite component were derived from the fit of a model to the atorvastatin only dataset. This choice of prior is supported by similar log P values for ATV and *o*-ATV and the limited structural difference between ATV and *o*-ATV, for example, *o*-ATV is formed via the addition of an OH group to the atorvastatin molecule.[33] The application of informative priors on selected parameters allowed the analysis to focus on the estimation of ATV CL/F by stabilizing the parts of the model that were not being informed by the sparse PK sampling scheme.

Using the BayesPrior method, the PK of ATV and *o*-ATV in pediatric patients with HeFH was described by a combined parent-metabolite model with two

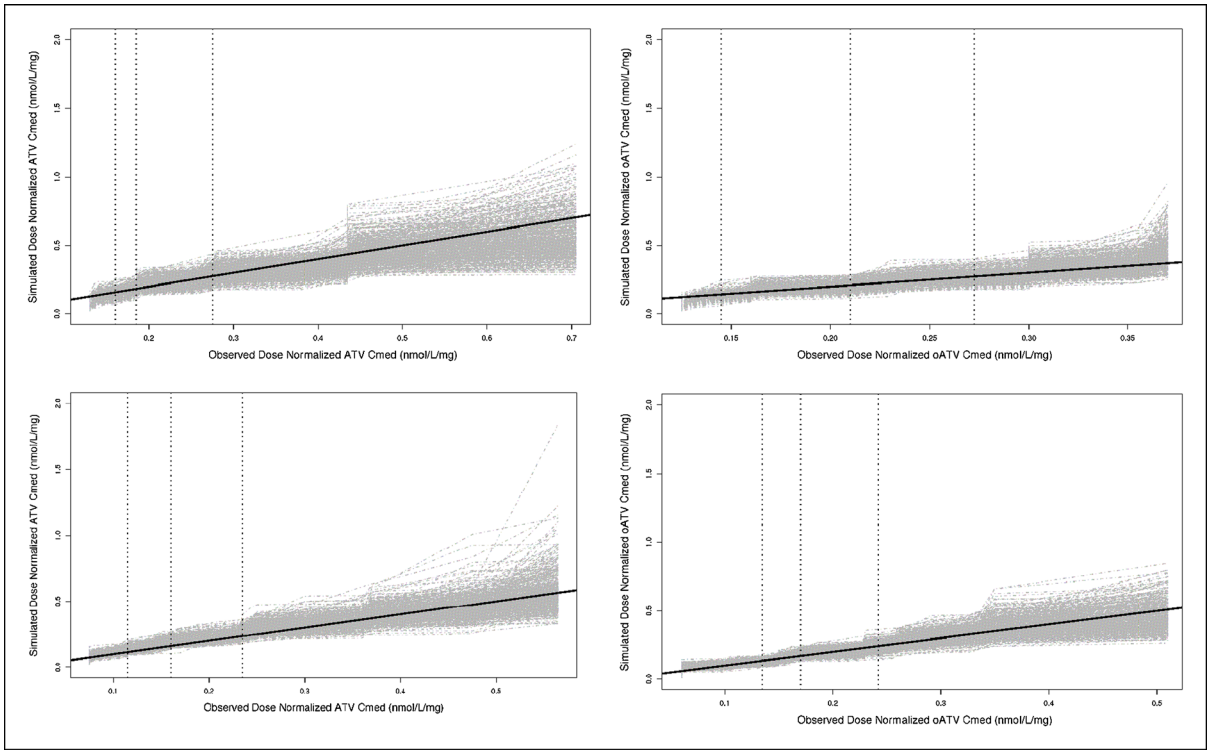

Figure 4. Distributions of simulated median dose normalized ATV and *o*-ATV concentrations within each individual (Cmed) for pediatric patients are compared to the actual observed distribution of Cmed values from the population PK database by Tanner Stage category. Quantile–quantile plots for each of the 500 simulation replicates are depicted by a gray dashed line and are overlaid on this plot. The black solid line represents a reference line of identity. The dotted vertical lines denote the location of the 1st quartile, median, and 3rd quartile of the observed data. Top: Tanner Stage 1, Bottom: Tanner Stage 2, Left: ATV, Right: *o*-ATV.

compartments for each component. In the final model, typical population PK parameters (95% CI) given the reference covariates (Tanner Stage $\geq$ 2, 70 kg weight) were 699 L/hour (570, 881), 1020 L (209, 2210), 227 L/hour (80.2, 470), 1960 L (1390, 2460), 0.200 $hour^{-1}$ (0.139, 0.304), 616 L/hour (518, 748), 401 L (272, 715), 368 L/hour (248, 562), 2040 L (1740, 2250), and 1 (fixed) for CL/F, Vc/F, Q/F, Vp/F, Ka, CLm/fm, Vcm/fm, Qm/fm, Vpm/fm, and F1, respectively. For those patients at Tanner Stage 1, the typical estimate (95% CI) for F1 was 0.752 (0.577, 1.01).

Overall the PK of atorvastatin in the subgroup of pediatric patients does not appear to be markedly dissimilar compared with adults. The typical CL/F (95% CI) for a 70 kg subject based on the final population PK model is estimated to be 699 L/hour (570, 881). The point estimate and entire 95% CI fall within the range of CL/F values calculated from the results of three recent PK studies of ATV in adults.[12–14] However, the results from a previous population PK analysis, following the multiple dose administration of ATV in adults, demonstrated a lower CL/F point estimate and 95% CI.[32] The difference in CL/F between these estimates can likely be attributed to differences in study design (primarily sparse vs. rich PK sampling schemes) and the overall variability in ATV PK rather than to differences specific to this subgroup of pediatric patients. While the study design and small sample size precludes any definitive statements, overall the PK of ATV in this subgroup of pediatric patients does not appear to be markedly dissimilar compared with adults.

The F1 estimate for Tanner Stage 1 in the final model revealed a 46% probability that the relative effect was at least 25% lower than the typical value for Tanner Stage $\geq$ 2, while the upper end of the probability density included the null value of one. The interpretation of this effect is unclear, in that the Tanner Stage classification in this analysis represents a composite of potential factors such as age, different atorvastatin doses, formulation, and/or treatment compliance. This mix of underlying characteristics is likely contributing to the poor precision of the parameter estimate. Formulation differences are unlikely due to the demonstrated bioequivalence between chewable tablets and marketed ATV tablets in adults.[12–14] In addition, physiologic or maturational changes in PK due to age are unlikely within the age range studied here.[34]

In interpreting the results of this analysis, it is important to realize the limitations of the model in terms of what is known about ATV. ATV undergoes extensive first-pass and systemic metabolism via cytochrome P4503A4. ATV has also been shown to be a substrate for several transport proteins including the P-glycoprotein efflux transporter

and hepatic uptake organic anion transporting polypeptide (OATP)1B1.[3,35] ATV and both its active hydroxyl acid metabolites undergo biotransformation to inactive atorvastatin lactone forms, which could occur nonenzmatically at low intestinal pH or via an acyl glucuronide intermediate pathway.[36,37] The current model does not account for these aspects of ATV disposition. This is primarily due to the lack of PK data collected during the absorption phase and the limited blood sampling scheme necessitated by the pediatric population. These aspects of the available data necessitated the use of a simpler model for ATV disposition. While the model does not describe all aspects of ATV disposition, it does provide reasonable estimates of ATV CL/F in pediatric patients with HeFH.

The ATV and *o*-ATV population PK modeling across this database of pediatric patients provided an analysis across a range of doses in 39 pediatric patients with HeFH. The selective use of informative prior information from adults was used to support portions of the model that were not well defined from the currently available pediatric data (Ka, Vp/F, Vcm/fm, Qm/fm, and Vpm/fm) and allowed for the estimation of the remaining parameters (CL/F, Vc/F, and CLm/fm) to be driven by the pediatric data alone. After accounting for the effect of body weight on CL/F, Vc/F, Q/F, Vp/F, CLm/fm, Vcm/fm, Qm/fm, and Vpm/fm using an allometric power model, the addition of other covariates resulted in little improvement of the model fit and in minimal decreases in unexplained interindividual variability.

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