

ORIGINAL ARTICLE

An integrated PK-PD model for cortisol and the 17-hydroxyprogesterone and androstenedione biomarkers in children with congenital adrenal hyperplasia

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Aims: The aim of this study was to characterize the pharmacokinetic/pharmacodynamic relationships of cortisol and the adrenal biomarkers 17-hydroxyprogesterone and androstenedione in children with congenital adrenal hyperplasia (CAH).

Methods: A nonlinear mixed-effect modelling approach was used to analyse cortisol, 17-hydroxyprogesterone and androstenedione concentrations obtained over 6 hours from children with CAH ($n = 50$). A circadian rhythm was evident and the model leveraged literature information on circadian rhythm in untreated children with CAH. Indirect response models were applied in which cortisol inhibited the production rate of all three compounds using an I_{max} model.

Results: Cortisol was characterized by a one-compartment model with apparent clearance and volume of distribution estimated at 22.9 L/h/70 kg and 41.1 L/70 kg, respectively. The IC_{50} values of cortisol concentrations for cortisol, 17-hydroxyprogesterone and androstenedione were estimated to be 1.36, 0.45 and 0.75 $\mu\text{g/dL}$, respectively. The inhibitory effect was found to be more potent on 17OHP than D4A, and the IC_{50} values were higher in salt-wasting subjects than simple virilizers. Production rates of cortisol, 17-hydroxyprogesterone and androstenedione were higher in simple-virilizer subjects. Half-lives of cortisol, 17-hydroxyprogesterone and androstenedione were 60, 47 and 77 minutes, respectively.

Conclusion: Rapidly changing biomarker responses to cortisol concentrations highlight that single measurements provide volatile information about a child's disease control. Our model closely captured observed cortisol, 17-hydroxyprogesterone and androstenedione concentrations. It can be used to predict concentrations over 24 hours and allows many novel exposure metrics to be calculated, e.g., AUC, AUC-above-threshold, time-within-range, etc. Our long-range goal is to uncover dose-

Mahmoud Al-Kofahi and Mariam A. Ahmed contributed equally in the conduct of this research. The authors confirm that the Principal Investigator for this study is Kyriakie Sarafoglou, and that she had direct clinical responsibility for patients.

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exposure–outcome relationships that clinicians can use in adjusting hydrocortisone dose and timing.

KEYWORDS

compartmental analysis, endocrinology, mathematical modelling, paediatric, population pharmacokinetics-pharmacodynamics, steroids

1 | INTRODUCTION

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is a rare form of adrenal insufficiency, characterized by decreased cortisol synthesis and excessive androgen production due to impairment of the negative feedback loop in the hypothalamic–pituitary–adrenal (HPA) axis between **cortisol** and **adrenocorticotrophic hormone (ACTH)**. Cortisol secretion has a circadian pattern: cortisol levels reach nadir at midnight, begin to rise around 0200, peak early in the morning and gradually decrease throughout the day.^{1,2} The circadian cortisol secretion pattern is derived from a dynamic ultradian rhythm of discrete cortisol pulses that follow circadian-driven ACTH pulses.^{1,3–5}

In patients with CAH, impaired endogenous cortisol production leads to increased ACTH-driven adrenal gland stimulation. In turn, this leads to excess production of **17-hydroxyprogesterone (17OHP)** and androgens such as **androstenedione (D4A)** and testosterone especially in the early morning hours.⁶ Depending on the severity of 21-hydroxylase deficiency, CAH is classified as either classic (severe phenotype) or non-classic (mild phenotype). The classic phenotype is further subdivided into simple-virilizing (SV) and salt-wasting (SW) based on whether there is adequate or deficient aldosterone production, respectively.

Patients with classic CAH require life-long glucocorticoid replacement and in children, **hydrocortisone (HC)** is the glucocorticoid of choice because of growth-suppressive effects of long-acting glucocorticoids.⁷ The goal of therapy is to suppress overproduction of androgens while avoiding the deleterious effects of glucocorticoid excess. The current CAH consensus treatment guideline of hydrocortisone 10–15 mg/m²/day in three divided doses⁷ exposes children to alternating states of chronic hyper- and hypo-cortisolemia with resultant hyperandrogenemia due to hydrocortisone's short half-life in children with CAH under 1 hour (range: 41–105 min).^{8–10} Further complicating treatment is large between-patient variability of cortisol pharmacokinetics (PK) and pharmacodynamic (PD) response to HC as measured by 17OHP and D4A.⁸

Response to HC therapy and disease control are evaluated by single measurements of 17OHP and/or D4A at clinic visits every 3–4 months along with clinical impressions.⁷ This monitoring practice provides limited information as single measurements only indicate whether control is apparently adequate at a single time point. Long-term outcome studies in children with CAH underscore the limitations of the current therapy and monitoring practices.^{11–14} The aim of the present study is to characterize the 6-hour time course of cortisol and

What is already known about this subject

- Children with CAH are treated with cortisol replacement yet still demonstrate adverse long-term disease outcomes.
- Current monitoring practices of single biomarker measurements provide limited disease control information due to the rapid half-lives of cortisol and adrenal steroids.
- A quantitative understanding of PK/PD relationships may lead to improved therapeutic outcomes.

What this study adds

- A system consisting of indirect response models and an underlying circadian rhythm closely predicts observed cortisol, 17-hydroxyprogesterone and androstenedione concentrations.
- Concentrations over 6 hours can predict concentrations over 24 hours under the model.
- The model provides the opportunity to explore exposure–outcome relationships in quantitative ways that have not been previously available.

the adrenal 17OHP and D4A biomarker responses following hydrocortisone dosing in children with CAH using a nonlinear mixed-effects modelling approach.

2 | METHODS

2.1 | Study population

This was a single-centre, open-label study approved by the Institutional Review Board (IRB) at the University of Minnesota. Patients were eligible for the study if they had a confirmed diagnosis of CAH based on hormonal and molecular testing, and receiving oral hydrocortisone as part of their care. Fifty subjects with classic CAH were studied. Subjects were on a variety of hydrocortisone maintenance dosing regimens. These regimens were based on clinical findings

TABLE 1 Summary of demographics

Characteristics	Median (range)	
Age (years)	7 (1.47–18.2)	
Weight (kg)	29 (10.8–80.6)	
Sex, n (%)	Male	27 (54.0)
	Female	23 (46.0)
Pubertal status, n (%)	Pre-pubertal ^a	39 (78)
	Pubertal	10 (20)
	Post-pubertal	1 (2)
Phenotype, n (%)	Salt-wasting	40 (80)
	Simple-virilizing	10 (20)
Morning dose (mg)	6.25 (1.25–12.5)	

^a7 of 39 pre-pubertal patients were having their puberty suppressed with histrelin acetate.

including growth velocity, weight gain, rate of bone age maturation, and adrenal steroid response as measured by 17OHP and D4A serum concentrations. Patient demographics and dose ranges are presented in Table 1.

2.2 | Drug administration, blood sampling and measurement methods

On the day before the study, patients received their usual evening hydrocortisone dose and were asked to fast after midnight. The following morning, patients were admitted to an outpatient clinical facility. A baseline pre-dose blood sample was obtained at 08:00 and the patients then received their regular oral HC morning dose of either tablet or an extemporaneously compounded suspension.¹⁵ Following the dose, serial blood samples were obtained at 15, 30, 45, 60, and 75 minutes, and at 1.5, 2, 2.5, 3, 4 and 6 hours. Patients had a standard breakfast 1 hour after the morning dose.

Samples were analysed by a validated assay for total cortisol, 17OHP and D4A concentrations using high performance liquid chromatography tandem mass spectrometry (LC–MS/MS) in Mayo Clinic Laboratories.¹⁶ The lower limit of quantification (LLOQ) for the total cortisol, 17OHP and D4A assays were 0.2 µg dL⁻¹, 40 ng dL⁻¹ and 15 ng dL⁻¹, respectively. The within- and inter-assay coefficients of variation (CV) were less than 10% and 15%, respectively.

2.3 | PK-PD modelling

Nonlinear mixed-effect modelling (NONMEM[®] 7.4; ICON Development Solutions, Ellicott City, MD, USA) was performed for all analyses using the first-order conditional estimation method with interaction. In all models described below, between-subject variabilities (BSV) were described by lognormal distributions. For the residual unexplained variability (RUV), additive and/or proportional error models were used. Final NONMEM[®] control streams for all analyses

are provided in the Supporting Information. Exploratory analyses and diagnostic graphics were performed using R 3.6.1 (The R Foundation for Statistical Computing) and RStudio 1.2.5001 (RStudio, PBC) and Perl-speaks-NONMEM (PsN 4.9.0, Uppsala, Sweden) under the Pirana[®] interface.¹⁷ A sequential PK-PD approach was employed in which a model for cortisol was first developed and the individualized empirical Bayes estimates (EBEs) of the cortisol PK parameters were placed into the dataset and their simulated concentrations used to drive the 17OHP and D4A biomarker responses (Figure 1). The 17OHP was analysed separately from the D4A as we assumed there was no dependency of one on the other.

2.4 | NONMEM data preparation

The NONMEM data set was prepared to allow the system to run for 48 hours to achieve steady state before fitting the 6-hour PK-PD data. Compartmental initial conditions were set to 0, while subjects received their daily hydrocortisone doses.

2.5 | Modelling of exogenous cortisol

To accommodate circadian rhythms, we chose to model endogenous cortisol and exogenously administered HC as two separate pools that were combined to yield the total cortisol concentration. The exogenous HC was modelled as a one-compartment model with first order elimination. Both cortisol oral clearance (CL/F) and volume of distribution (V/F) were modelled using standard weight-based allometric scaling. Only three subjects were less than 2 years of age and no attempt was made to identify a maturation factor. Oral hydrocortisone was assumed to enter from a depot compartment using an Erlang absorption model.¹⁸ A sensitivity analysis-determined series of four transit compartments adequately described the distributed-delay absorption process.

2.6 | Modelling endogenous circadian cortisol

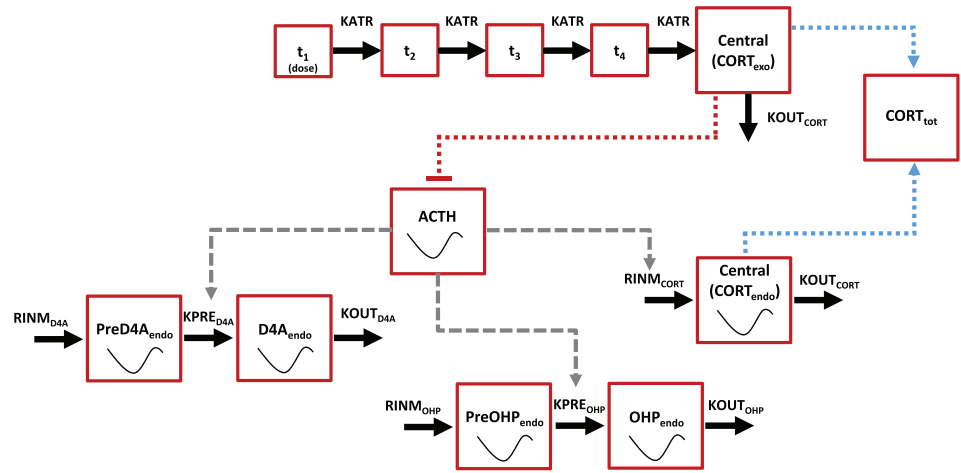
The endogenous cortisol was modelled as an indirect response model with zero-order production and first-order elimination rates.¹⁹ The production rates were allometrically scaled and the clearances and volume of distributions were shared between the endogenous and exogenous compartments.

The observation that many subjects demonstrated cortisol concentrations that were lower at the end of the study (14:00) compared to baseline concentration (08:00) values dictated that the endogenous production rate is not constant but decreasing over 6 hours. This is consistent with the known circadian rhythm of the HPA axis²⁰ in healthy volunteers as well as children with CAH.^{21–24}

ACTH is pivotal in regulating adrenocortical circadian rhythms, and is itself responsive to a complex set of underlying physiological mechanisms and feedback pathways.^{25–29} We chose to model a

FIGURE 1

Pharmacokinetic/pharmacodynamic model of cortisol, 17OHP and D4A. The PK are described by a one-compartment model with first order elimination in which concentration in the central compartment (C_p) is linked to the PD model. Oral hydrocortisone was assumed to enter from a depot compartment using an Erlang absorption model. Endogenous cortisol, 17OHP and D4A were described by indirect response models with zero-order production and first-order elimination



circadian process that is directly responsible for regulating endogenous production rates of cortisol (as well as 17OHP and D4A) and is also subject to inhibition by cortisol concentration. For simplicity, we chose to conceptualize this process as representing ACTH and refer to it as such. However, ACTH concentrations were not obtained and this was strictly a conceptualization of convenience that provides a relevant physiologic context to the model.

A dual-cosine function was used to describe an asymmetric circadian profile of ACTH,³⁰ according to the following:

If the time is between 0 and t_{\min} :

$$ACTH_{circ} = ACTH_{mean} + ACTH_{AMP} \times \cos\left(\frac{2\pi \times (t + 24 - t_{\max})}{(t_{\min} - t_{\max} + 24)}\right)$$

If time is between t_{\min} and t_{\max} :

$$ACTH_{circ} = ACTH_{mean} + ACTH_{AMP} \times \cos\left(\frac{2\pi \times (t - (2 \times t_{\min}) + t_{\max})}{(t_{\max} - t_{\min})}\right)$$

If time is between t_{\max} and 24 hours:

$$ACTH_{circ} = ACTH_{mean} + ACTH_{AMP} \times \cos\left(\frac{2\pi \times (t - t_{\max})}{(t_{\min} - t_{\max} + 24)}\right)$$

where $ACTH_{mean}$ is the mean 24-hour concentration; $ACTH_{AMP}$ is distance from the mean concentration to the maximum or minimum concentrations; t is the time; t_{\max} and t_{\min} are the time where the maximum concentration and minimum concentration occurs, respectively.

However, since our study has data collected over only 6 hours, insufficient information was available to support estimation of parameters that describe a 24-hour cycle. To that end, we leveraged existing literature to model ACTH driving force parameters from cortisol and 17OHP circadian rhythms.

Frisch et al. reported 24-hour endogenous cortisol and 17OHP concentrations in children with CAH not receiving glucocorticoid therapy for at least 3 days.²¹ WebPlotDigitizer 3.3 was used to extract the data.³¹ While separate indirect response models were proposed to describe endogenous cortisol and 17OHP concentrations, the

parameters of a single dual-cosine function were used to describe an ACTH circadian rhythm pattern that regulated the production of cortisol and 17OHP. The $ACTH_{mean}$ parameter was fixed to unity and the estimated circadian profile of ACTH was converted to a fractional change by normalizing ACTH to the maximum value ($1 + ACTH_{AMP}$), according to the following:

$$ACTH_{max} = ACTH_{mean} \times (1 + ACTH_{AMP})$$

$$ACTH_{normalized} = \frac{ACTH_{circ}}{ACTH_{max}}$$

where $ACTH_{max}$ is maximum ACTH value and $ACTH_{normalized}$ is the normalized ACTH to the maximum value.

2.7 | Linking endogenous cortisol production and oral hydrocortisone dosing

The population-level $ACTH_{AMP}$, t_{\max} and t_{\min} parameters of this dual cosine function were brought forward in the analysis of cortisol (and 17OHP and D4A) concentrations following oral HC to inform the underlying circadian rhythm in the absence of exogenous HC under the assumption that $ACTH_{AMP}$, t_{\max} and t_{\min} were similar in treated and untreated children with CAH.

Cortisol itself exerts a negative feedback on the HPA axis and reduces cortisol production rate in healthy people via inhibiting ACTH.^{20,27,28,32} We assumed the endogenous normalized ACTH function has cortisol inhibition built into it through the circadian rhythm and attributed additional inhibition to exogenous hydrocortisone only. This additional inhibition was modelled using a sigmoid I_{\max} model that inhibited the production of the normalized ACTH, according to the following:

$$f(\text{drug}) = \frac{I_{\max} \times Cort_{exo}^h}{IC_{50}^h + Cort_{exo}^h}$$

$$ACTH_{inhibited} = ACTH_{normalized}(1 - f(\text{drug}))$$

$$\frac{dCort_{endo}}{dt} = Rin_{cort} \times ACTH_{inhibited} - K_{out} \times Cort_{endo}$$

where $f(\text{drug})$ is the drug effect that is a function of the drug concentration; I_{max} is the maximum inhibition effect of the drug; $Cort_{exo}$ is the exogenous cortisol concentration; IC_{50} is the added exogenous concentration at which 50% of drug effect occurs; h is hill coefficient; $ACTH_{inhibited}$ is the inhibited production of the normalized ACTH; $Cort_{endo}$ is the endogenous cortisol concentration; Rin_{cort} is the zero-order rate constant for endogenous cortisol production in the absence of exogenous cortisol; and K_{out} is the first-order rate constant for dissipation of endogenous cortisol.

It was assumed that cortisol could completely inhibit ACTH at high concentrations with $I_{max} = 1$. The exogenous cortisol concentration that inhibits the normalized ACTH by 50% (IC_{50}) was estimated. A sensitivity analysis led to the sigmoidicity factor (h) being fixed to 3.0. This inhibitory function was used to suppress the circadian ACTH function. Hence, the endogenous cortisol production rate is a function of the circadian ACTH pattern, which in turn was inhibited by exogenous cortisol concentrations.

2.8 | Precursor-dependent PD modelling of 17OHP and D4A

The individualized cortisol EBE parameters from the cortisol modelling were fixed and made available in the data set for the 17OHP and D4A modelling. The predicted exogenous cortisol concentrations were assumed to inhibit the HPA axis as described above for cortisol, but the parameters of the 17OHP and D4A I_{max} models were estimated uniquely for each compound under the assumption that the 17OHP and D4A production may respond differentially to exogenous cortisol.

In the PD modelling of the 17OHP and D4A biomarkers, a precursor compartment was needed for the 17OHP and D4A indirect response models,³³ according to the following:

$$\frac{dPre_{OHP/D4A}}{dt} = Rin_{O(OHP/D4A)} - K_{preOHP/D4A} \times ACTH_{inhibited(OHP/D4A)} \times Pre_{OHP/D4A}$$

$$\frac{dR_{OHP/D4A}}{dt} = K_{preOHP/D4A} \times ACTH_{inhibited(OHP/D4A)} \times Pre - K_{outOHP/D4A} \times R_{OHP/D4A}$$

where $R_{OHP/D4A}$ is the observed response; Rin_0 represents the zero-order rate constant for precursor production in the absence of exogenous cortisol and expressed in terms of concentration per time; K_{pre} is the first-order rate constant for production of the response from the precursor; and K_{out} is the first-order rate constant for dissipation of response.

2.9 | Model evaluations

The precision of the final model parameters were evaluated using sampling-importance-resampling (SIR)-based 95% confidence intervals (CIs).^{34,35} The final models were evaluated using prediction-corrected visual predictive checks (pcVPC; 1,000 simulations).³⁶

2.10 | Evaluating the predictive ability of PKPD parameters

For the model to be considered fit for purpose, we determined if analysis of the data from 6 hours were able to predict cortisol, 17OHP and D4A concentrations over 24 hours. One paediatric patient with CAH had 24-hour cortisol, 17OHP and D4A profiles obtained under a separate IRB approved study protocol (R01FDR0006100) following doses at times 0, 6 and 12 hours. A total of 25 concentrations were obtained with eight of those obtained during the first 6 hours. The model described above was fit to data from the first 6 hours. These parameters were then used to simulate the concentration-time profiles over 24 hours. The predictive ability of the estimated parameters from the 6-hour analysis was assessed by overlaying the observations from 24 hours on top of the predicted 24-hour concentration-time profile. In addition, the observed and simulated AUC_{0-6} , AUC_{6-12} and AUC_{12-24} were quantitatively evaluated from observed and predicted concentrations at those time points. Relative AUC deviation (%) was calculated as: $(AUC_{PRED} - AUC_{OBSERVED})/AUC_{OBSERVED}$. Given the dynamic equilibrium of the HPA axis and the uncertainty in the modelling of the negative feedback loops, we considered a 40% deviation as a reasonable and a 10% deviation as an excellent prediction.

This study was approved by the University of Minnesota institutional review board and was conducted in accordance with the current revision of the Declaration of Helsinki.

2.11 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY.³⁷

3 | RESULTS

3.1 | Data

Four subjects demonstrated persistently undetectable 17OHP and D4A concentrations and were excluded from the analysis of 17OHP and D4A. An additional eight subjects showed persistently undetectable D4A concentrations (but measurable 17OHP concentrations) and were excluded from the analysis of D4A. Overall

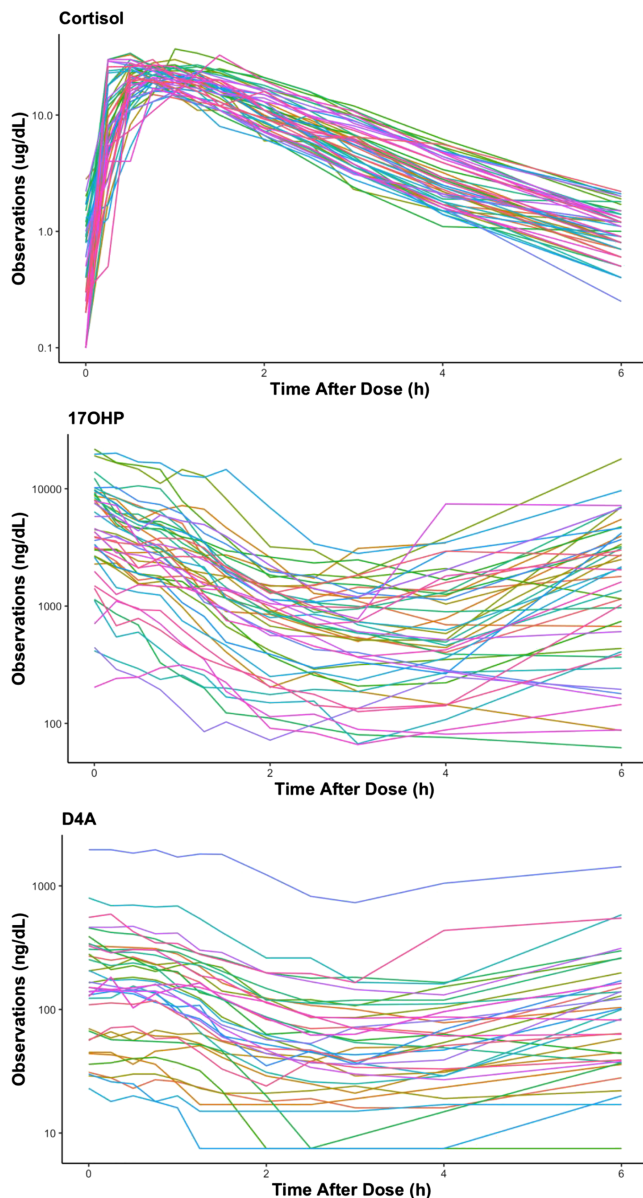


FIGURE 2 Spaghetti plot of individual plasma profiles. Lines represent the observed plasma concentrations of cortisol, 17OHP and D4A. Top panel: Cortisol plasma concentrations. Middle panel: 17OHP plasma concentrations. Bottom panel: D4A plasma concentrations

592 cortisol samples from 50 subjects, 545 17OHP samples from 46 subjects and 438 D4A samples from 38 subjects were included in the analyses. After excluding the 17OHP and D4A concentration profiles that were persistently undetectable, occasional concentrations of cortisol, 17OH and D4A were below the limit of quantification. These values were imputed with one-half the LLOQ. The percentages of data below the limit of quantification were 1.3% for cortisol, 0.2% for 17OHP and 1.8% for D4A. Spaghetti plots of individual plasma concentrations of cortisol, 17OHP and D4A are shown in Figure 2.

3.2 | PK-PD model

3.2.1 | Endogenous cortisol modelling in untreated CAH children

The dual-cosine model estimated the $ACTH_{AMP}$ at 46% of the mesor (fixed to 1.0) and the times at which the maximum (t_{max}) and the minimum (t_{min}) production rates occur at 07:05 and 22:55, respectively.

3.2.2 | PK-PD model parameters

After including allometrically scaled CL/F and V/F using body weight, it was found that disease phenotype was a significant determinant of cortisol production rate. The estimated typical values for CL/F and V/F are for a standard 70 kg adult. For a standard 29 kg child, the CL/F and V/F values were calculated to be 11.7 L/h and 17.0 L, respectively. Similarly, after allometrically scaling 17OHP and D4A production rates by body weight, a significant improvement to the model was realized with the inclusion of disease phenotype. The IC_{50} of exogenous cortisol required to reduce 17OHP and D4A production rate by 50% was estimated to be 0.45 $\mu\text{g/dL}$ and 0.75 $\mu\text{g/dL}$, respectively. As shown in Figure 3, IC_{50} values for D4A tend to be higher than those for 17OHP, and this effect persists in both SV and SW subjects. Moreover, SW subjects tend to have higher IC_{50} values for both 17OHP and D4A. Age and sex were not found to have any statistically significant ($P > 0.001$) effect on these parameters. The final PK model parameter estimates, their relative standard error (RSE), η and ϵ shrinkage, and SIR-based 95% CIs for cortisol, 17OHP and D4A are provided in Tables 2, 3 and 4, respectively.

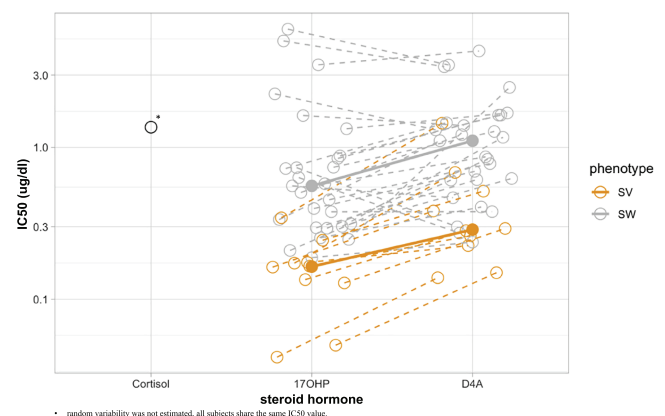


FIGURE 3 IC_{50} values for cortisol, 17OHP and D4A in SW and SV subjects plotted on a log scale. Left column: IC_{50} values for cortisol. Middle column: IC_{50} values for 17OHP. Right column: IC_{50} values for D4A. Open black circles correspond to the IC_{50} values for cortisol in subjects with both SW and SV. Open grey circles correspond to the IC_{50} values for 17OHP and D4A in subjects with SW, and solid grey circles correspond to the median IC_{50} values. Open orange circles correspond to IC_{50} values for 17OHP and D4A in subjects with SV, and solid orange circles correspond to the median IC_{50} values. Dashed lines connect the IC_{50} values for 17OHP and D4A for each subject, and solid lines connect the median IC_{50} values

TABLE 2 Final cortisol PK parameter estimates, precisions and shrinkages

Parameter (unit)	Estimate (relative standard error %)	SIR median (95% confidence interval)	Shrinkage % ^a	Notes
RINM _{CORT} (mg h ⁻¹)				RINM _{CORT} , mean endogenous production rate of endogenous cortisol
Salt-wasting group	0.066 (WT/70) ^{0.75} (16.9)	0.067 (0.049–0.089)		WT, weight in kg
Simple-virilizing group	0.336 (WT/70) ^{0.75} (18.1)	0.337 (0.198–0.486)		
Oral hydrocortisone PK parameters				
CL/F (L h ⁻¹)	22.9 (WT/70) ^{0.75} (4.2)	22.9 (21.4–24.8)		CL/F, apparent clearance
V/F (L)	41.1 (WT/70) ^{1.0} (3.5)	41.1 (38.7–43.6)		V/F, apparent volume of distribution
KATR (h ⁻¹)	10.4 (5.8)	10.4 (9.3–11.6)		KATR, transit rate constant associated with transit absorption model
Effect of hydrocortisone on endogenous cortisol production rate				
IC ₅₀ (ug dL ⁻¹)	1.36 (17.7)	1.41 (1–1.88)		IC ₅₀ , concentration of cortisol resulting from exogenous hydrocortisone dosing required to produce 50% inhibition of the maximum effect on endogenous cortisol production rate
γ	3 (fixed)			γ a hill coefficient
Between-subject variability, coefficient of variation%				Coefficient of variation % was calculated as $\sqrt{e^{\omega^2} - 1}$
RINM _{CORT}	120.6 (22.4)	125.6 (92.6–167.9)	5.5	
CL/F	28.5 (18.6)	29.2 (24.1–34.1)	1.3	
V/F	20.4 (33.5)	21.4 (16.6–26.8)	10.3	
KATR	40.4 (21.1)	41.4 (33.4–49.1)	5	
Residual unexplained variability				
Proportional, CCV% and (SD)	19.2 (13)	19.3 (17.9–21)	14.6	CCV%, constant coefficient of variation% and SD, standard deviation were calculated as $\sqrt{\sigma^2}$

^aShrinkage was calculated for both η and ϵ as shown by Savic et al.⁵⁴

3.2.3 | Model evaluations

The overall goodness-of-fit plots indicated little reason to reject the model (Supporting Information). The pcVPC are shown in Figure 4 on a log scale. The prediction-corrected observed 5th, 50th and 95th data percentiles for most time bins were within the predictive intervals of prediction-corrected simulations. The final model parameter estimates were all within their SIR-based 95% CIs (Tables 2–4).

3.2.4 | Evaluating the stability of PKPD parameters over 24 hours

The 24-hour concentration–time profiles for cortisol, 17OHP and D4A predicted from the morning dose 6-hour data, along with the observed concentrations, are presented in Figure 5. Relative to the observed AUCs following each dose (0–6, 6–12 and 12–24 h), the percent differences for each dosing interval for each compound are presented

in Table 5 with seven of the nine values having a discrepancy less than 20%.

4 | DISCUSSION

In the current analysis, a nonlinear mixed-effects modelling approach was applied to develop a model that captured the interdependence of hydrocortisone dosing and the cortisol, 17OHP and D4A concentration–time profiles in children with CAH. This model included a negative feedback loop of cortisol that suppressed the drive of the endogenous circadian rhythm. The administration of hydrocortisone decreased the endogenous production rate of cortisol, 17OHP and D4A, and the inhibition of production decreased as the exogenous cortisol was eliminated from the body.

In our 6-hour PKPD study, the underlying circadian rhythm of cortisol was apparent. Notably, the baseline cortisol concentration at 08:00 was higher than cortisol concentration at 14:00 in most

TABLE 3 Final 17-hydroxyprogesterone (17OHP) parameter estimates, precisions and shrinkages

Parameter (unit)	Estimate (relative standard error %)	SIR median (95% confidence interval)	Shrinkage % ^a	Notes
17-hydroxyprogesterone (17OHP) indirect response model parameter estimates				
$k_{out17OHP}$ (h ⁻¹)	0.88 (4.3)	0.88 (0.82–0.95)		$k_{out17OHP}$, elimination rate constant of 17OHP
$k_{pre17OHP}$ (h ⁻¹)	0.71 (31.8)	0.73 (0.48–1.08)		$k_{pre17OHP}$, first-order rate constant for production of the response from the 17OHP precursor
$RINM_{17OHP}$ (ng dL ⁻¹ h ⁻¹)				$RINM_{17OHP}$, mean endogenous production rate of endogenous cortisol
Salt-wasting group	3,780 (WT/70) ^{0.75} (19.1)	3,829 (2,684–5,253)		WT, weight in kg
Simple virilizing group	5,020 (WT/70) ^{0.75} (13.9)	5,032 (3,579–6,487)		
Effect of hydrocortisone on 17-hydroxyprogesterone (17OHP) production rate				
IC ₅₀ (μg dL ⁻¹)	0.45 (58.5)	0.45 (0.2–0.78)		IC ₅₀ , concentration of cortisol resulting from exogenous hydrocortisone dosing required to produce 50% inhibition of the maximum effect on endogenous 17OHP production rate
γ	1.22 (16.6)	1.22 (1.02–1.43)		γ , a hill coefficient
Between-subject variability, coefficient of variation%				Coefficient of variation was % was calculated as $\sqrt{e^{\omega^2} - 1}$
$RINM_{17OHP}$	149.1 (10.6)	152.4 (109–215.2)	1.4	
$K_{out17OHP}$	22.3 (17.2)	23.3 (17–29.8)	11.8	
IC ₅₀	176 (16.7)	182.8 (121.2–291.6)	4.3	
Residual unexplained variability				
Proportional, CCV% and (SD)	23.9 (8.9)	24.1 (22.3–25.9)	10.4	CCV%, constant coefficient of variation% and SD, standard deviation were calculated as $\sqrt{\sigma^2}$
Additive, SD FIXED	0.0001			

^aShrinkage was calculated for both η and ϵ as shown by Savic et al.⁵⁴

subjects. It can be assumed that the 08:00 cortisol concentration must be a result of endogenous cortisol production as it was obtained approximately 12 half-lives after the previous evening dose. A lower concentration 6 hours after a morning dose can be explained by assuming the cortisol production rate at 14:00 is lower than at 08:00. This latter assumption is supported by the known circadian rhythm that exists in children with CAH^{21,22} and is more plausible than assuming cortisol clearance is increasing over 6 hours. Hence, we leveraged literature data to develop a dual-cosine model describing a 24-hour circadian pattern.

The cortisol PK model estimated CL/F of cortisol for CAH patients to be 22.9 L h⁻¹ for a standard 70-kg person. This is somewhat higher than that for healthy subjects or for patients with other forms of adrenal insufficiency.^{37,38} There are several possibilities for this. Allometric scaling of clearance to body weight may not fully hold across the ages of children we studied. Perhaps the physiological ramifications of CAH, including fluctuating adrenal androgen levels over

the course of 24 hours, may cause a change in cortisol clearance by altering the enzyme activity of 11 β -hydroxysteroid dehydrogenase (11 β -HSD) isoenzymes and other enzymes that have a role in cortisol clearance.^{39–41} However, it is also possible that the differences are due to methodological differences. Unlike most other PK studies, our analysis did not rely on the dexamethasone suppression test and the assumption that endogenous ACTH and cortisol production rates were fully suppressed to zero. The estimated daily endogenous cortisol production rates in SW and SV were 0.034 mg h⁻¹ and 0.173 mg h⁻¹, respectively, which is lower than that reported in healthy children of 0.396 mg h⁻¹.⁴² In addition, although endogenous cortisol production is low in children with CAH, it is not fully absent and ignoring endogenous concentrations will lead to a systematic overestimation of the half-life and AUC when calculated by the trapezoidal rule in a noncompartmental analysis. This results in the underestimation of CL/F values. V/F in CAH patients (41.1 L 70Kg⁻¹) is similar to that reported for individuals with other forms of adrenal

TABLE 4 Final androstenedione (D4A) parameter estimates, precisions and shrinkages

Parameter (unit)	Estimate (relative standard error %)	SIR median (95% confidence interval)	Shrinkage % ^a	Notes
Androstenedione (D4A) indirect response model parameter estimates				
K_{outD4A} (h^{-1})	0.54 (6.2)	0.54 (0.47–0.6)		K_{outD4A} , elimination rate constant of D4A
k_{preD4A} (h^{-1})	0.74 (19.2)	0.75 (0.55–1.02)		k_{preD4A} , first-order rate constant for production of the response from the D4A precursor
$RINM_{D4A}$ ($ng\ dL^{-1}\ h^{-1}$)				$RINM_{D4A}$, zero-order rate constant for precursor production in the absence of exogenous cortisol
Salt-wasting group	97.6 (WT/70) ^{0.75} (20.7)	98.93 (67.56–133.41)		WT, weight in kg
Simple-virilizing group	118 (WT/70) ^{0.75} (17.6)	116.69 (72.47–162.94)		
Effect of hydrocortisone on androstenedione (D4A) production rate				
IC_{50} ($ug\ dL^{-1}$)	0.75 (27.1)	0.76 (0.46–1.11)		IC_{50} , concentration of cortisol resulting from exogenous hydrocortisone dosing required to produce 50% inhibition of the maximum effect on endogenous D4A production rate
γ	1 (fixed)			γ : a hill coefficient
Between-subject variability, coefficient of variation %				Coefficient of variation % was calculated as $\sqrt{e^{\sigma^2} - 1}$
$RINM_{D4A}$	108.5 (23.7)	115.5 (85.5–154.4)	2.5	
K_{outD4A}	23.2 (41.7)	24.5 (16.7–32.4)	16.4	
IC_{50}	120.3 (26.8)	122.9 (84.6–174.1)	7.6	
Residual unexplained variability				
Proportional, CCV% and SD	17.5 (11.9)	17.5 (16.1–19)	11.1	CCV%, constant coefficient of variation% and SD, standard deviation were calculated as $\sqrt{\sigma^2}$

^aShrinkage was calculated for both η and ϵ as shown by Savic et al.⁵⁴

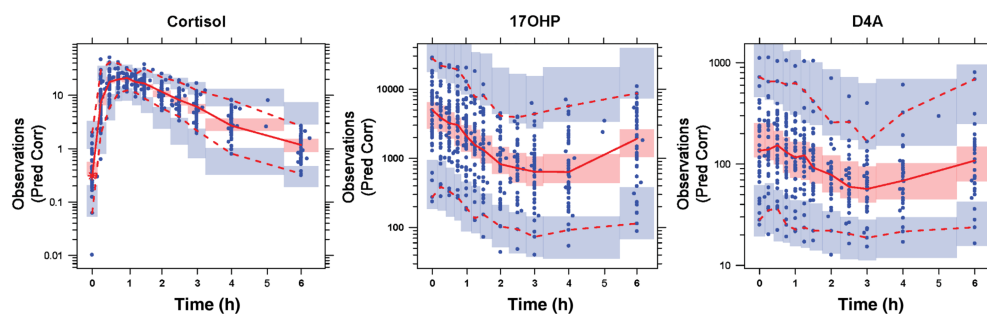


FIGURE 4 Log prediction-corrected (pc) visual predictive check (pcVPC). Lines represent the 5th, 50th and 95th percentiles of the pc observations; and shaded areas represent 95% prediction intervals of the 5th, 50th and 95th percentiles of pc simulated data. Left panel: Cortisol pcVPC. Middle panel: 17OHP pcVPC. Right panel: D4A pcVPC

insufficiency (38.7 L) or healthy volunteers (32 L) following an intravenous dose.^{38,43}

Since 17OHP and D4A were not administered directly, our study design was not able to estimate clearances and volume of distributions. Nonetheless, the model did quantify the half-lives of these biomarkers and found them to be rapid at 47 and 77 minutes for 17OHP and D4A, respectively. The differences in IC_{50} values between 17OHP and D4A suggest that cortisol is a more potent inhibitor of 17OHP than D4A production. Clinically, this means that for a given

cortisol concentration, the 17OHP and D4A biomarker responses may differ, with 17OHP being more responsive than D4A.

Recently, Melin et al.⁴⁴ described a similar type of PKPD analysis in children with CAH. But unlike ours, it only analysed cortisol and 17OHP and did not include analysis of D4A concentrations, the primary indicator for adrenal androgen control as D4A is associated with virilization, short stature due to advanced bone age and early epiphyseal closure, precocious puberty, polycystic ovarian syndrome, infertility and metabolic syndrome, to name a few.^{11–14,45–47} Another major

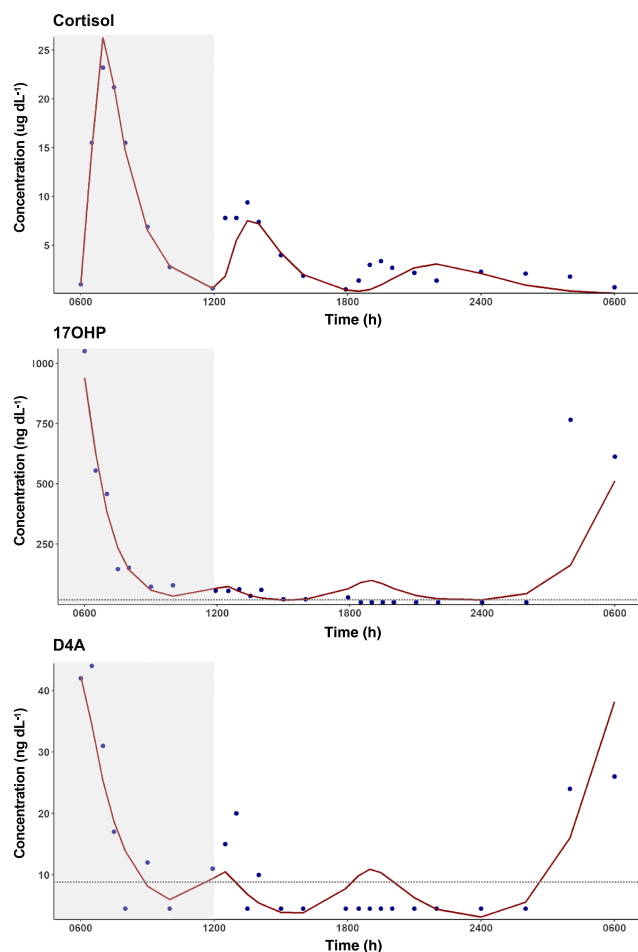


FIGURE 5 Observed vs simulated cortisol, 17OHP and D4A 24-h concentrations for one paediatric patient. The 24-h concentration-time profiles for cortisol, 17OHP and D4A predicted (solid lines) from the morning dose 6-h data, along with the observed concentrations (closed circles). Dashed line represents LLOQ. Top panel: Cortisol. Middle panel: 17OHP. Bottom panel: D4A

TABLE 5 Relative AUC deviation (%)

Compound	Time interval (h)	Relative AUC deviation (%) ^a
Cortisol		
	0–6	1.9
	6–12	–19.7
	12–24	–30.6
17-hydroxyprogesterone		
	0–6	5.1
	6–12	–6.8
	12–24	44.5
Androstenedione		
	0–6	1.9
	6–12	–20.0
	12–24	9.3

^aRelative AUC deviation (%) calculated as: $(AUC_{\text{PRED}} - AUC_{\text{OBSERVED}}) / AUC_{\text{OBSERVED}}$.

difference is in the assumptions used to create our respective models. Melin et al. assumed endogenous cortisol production was constant over 24 hours, an important assumption given data were collected over 24 hours.⁴⁴ However, this is in contrast to what has been described previously in the literature^{21,22,48} and to our observations that clearly demonstrated the need to model cortisol production as a decreasing function over 6 hours. While all cortisol PK parameters are remarkably similar between the two analyses, this difference in baseline cortisol production rate is likely a major contributor to differences in 17OHP parameters. The 17OHP IC_{50} parameters between the two reports are difficult to compare as Melin et al. assume the IC_{50} parameter reflects total cortisol concentrations, whereas under our circadian rhythm assumptions, IC_{50} is the additional cortisol concentration due to exogenous HC administration, i.e., above the endogenous concentration. As expected, our 17OHP IC_{50} value is smaller, but when added to our typical endogenous baseline cortisol concentration, the values are similar with 1.33 $\mu\text{g}/\text{dL}$ compared to the 1.76 $\mu\text{g}/\text{dL}$ in the Melin et al. study. Comparisons of 17OHP production rates between the two studies are also difficult to compare. Based on Melin et al., 17OHP baseline and elimination values, one can calculate their input rate to be approximately 569 $\text{ng}/\text{dL}/\text{hr}$, but that is estimated in the presence of cortisol inhibition. With our assumptions, the production rate is approximately 4,400 $\text{ng}/\text{dL}/\text{hr}$ and that assumes there is no inhibition from endogenous cortisol until exogenous HC is administered. Nonetheless, our predicted baseline 17OHP concentration of about 5,000 ng/dL is consistent with the data presented in our Figure 2, while the Melin et al. value of 38.6 nmol/L (1,276 ng/dL) appears to be consistent with their presented data. The Melin et al. value for the 17OHP K_{out} of 0.446 hr^{-1} is smaller than the corresponding value of 0.88 hr^{-1} we estimated. The corresponding half-lives are 1.6 hour vs 0.79 hour. In our observed 17OHP data, it can be seen that most subjects have reached a minimum concentration in 3 or 4 hours, similar to the findings in Fuqua et al.⁴⁹ This rapid fall off in 17OHP concentration is consistent with a rapid half-life and is more plausible than the 1.6 hour value. It could be the case that the Melin et al. data do support a longer half-life than we estimate, but it is impossible to tell from the display in their figure 1 due to the compressed time scale and the presentation of medians and inter-quartile ranges at every 20-minute time intervals, or in their figure 3 VPC because of comingling the twice- and thrice-daily dosing data. Finally, some of the parameter differences could be attributed to differences between the radioimmunoassay used by Melin et al. and the LC-MS/MS assay we employed.

Given the complexity of the models, it is not surprising that deviations exist between observed and predicted concentrations over 24 hours. While we only had 24-hour data from a single child with CAH, it is encouraging that the cortisol, 17OHP and D4A partial AUC deviations between observed values and those predicted from a 6-hour profile range from 1.9–30.6%, 5.1–44.5% and 1.9–20.0%, respectively. Although the last dosing interval of 17OHP showed a higher relative AUC deviation, the predicted and observed concentrations were all low until the 17OHP rebounded as exogenous cortisol was cleared from the body. Overall, visual inspection clearly

showed that the model has an acceptable prediction performance over 24 hours.

One limitation of the study was that there was insufficient data available in our 6-hour PK-PD profiles to describe endogenous adrenal steroid circadian rhythm and several assumptions were needed to capture our observed data. Evidence of a circadian rhythm in untreated children with CAH is compelling,^{21,22,48,50-52} but a study design and analysis using stable-labelled hydrocortisone to demonstrate its existence definitively would be needed. Our limited 6-hour data did dictate the need for an endogenous circadian rhythm and the inclusion of endogenous and exogenous cortisol sources fit our cortisol data closely.

Optimizing cortisol replacement therapy in children with CAH remains challenging due to the complex underlying physiological mechanisms regulating a dynamic HPA axis, adrenal steroid circadian rhythms and rapid half-lives. Once it is understood how rapidly cortisol, 17OHP and D4A change in response to hydrocortisone therapy, the recommendation to monitor therapeutic response with a single measurement every 3 to 4 months⁷ appears limited. It is unlikely that a single cortisol, 17OHP or D4A concentration obtained at any point in time will be a useful metric for correlation with outcomes. The ability to obtain frequent samples over 24 hours is possible⁵³ but the technology is not universally available. However, shorter 6-hour PK studies can be conducted at many centres. We have evidence in one child that this model and 6 hours of data can predict concentrations of cortisol, 17OHP and D4A over the remaining 18 hours of the day. It is important to note that this model can be used to predict a continuum of concentration–time profiles and derive many exposure metrics. Simply keeping concentrations within a given range of values based on unaffected children may be a good first step, but it is not sufficient. What is critically needed in the field is evidence that any of these targets, whether single concentrations, AUCs or time within a range, etc., are associated with positive or negative long-term outcomes. We cannot rule out the possibility that perhaps higher or lower metrics compared to healthy controls might be needed for optimal results in children with CAH. Evidence of the gap in knowledge that still exists with current treatment and monitoring is reflected through many non-quantitative statements in the current Endocrine Society CAH Consensus guidelines⁷: “Adjusting medications for CAH is difficult.” “We do not provide specific target levels for adrenal steroid measurements.” “Clinicians should adjust doses in the context of the overall clinical picture and not solely based on a single laboratory assessment.” The CAH Consensus guidelines reflect informed opinions on the best practices available, but the limitations in the current state of knowledge points to the need for new insights into treatment and monitoring to optimize patient care with evidence-based information.

5 | CONCLUSION

The model we developed assumes a circadian rhythm of ACTH and closely captures observed cortisol, 17OHP and D4A concentrations in children with CAH being treated with hydrocortisone. It is important that just 6 hours of data may be able to reasonably predict 24-hour

profiles as it simplifies our ability to capture a variety of novel exposure metrics in the endocrine field. Ongoing and future work with this model involves conducting rigorous studies of dose–exposure–outcome relationships to determine which exposure metrics are related to which long-term outcomes.

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COMPETING INTERESTS

There are no competing interests to declare.

CONTRIBUTORS

M.A.A., M.A., R.C.B. and K.S. wrote the manuscript. C.L.Z. and K.S. designed the research. M.T.G.B. and K.S. performed the research. B.A.W., M.A.A., M.A., M.M.J, T.N.T., R.C.B. and K.S. analysed the data.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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