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The circadian rhythm of corticosteroid-binding globulin has little impact on cortisol exposure after hydrocortisone dosing

Short title: Impact of circadian CBG on cortisol exposure

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Summary

Context: Optimisation of hydrocortisone replacement therapy is important to prevent under- and over dosing. Hydrocortisone pharmacokinetics is complex as circulating cortisol is protein bound mainly to corticosteroid-binding globulin (CBG) that has a circadian rhythm.

Objective*:* A detailed analysis of the CBG circadian rhythm and its impact on cortisol exposure after hydrocortisone administration.

Design and Methods: CBG was measured over 24 h in 14 healthy individuals and, employing a modelling and simulation approach using a semi-mechanistic hydrocortisone pharmacokinetic model, we evaluated the impact on cortisol exposure (area under concentration-time curve and maximum concentration of total cortisol) of hydrocortisone administration at different clock times and of the changing CBG concentrations.

Results: The circadian rhythm of CBG was well described with two cosine terms added to the baseline of CBG: baseline CBG was 21.8 µg/mL and inter-individual variability 11.9%; the amplitude for the 24 h and 12 h cosine functions were relatively small (24 h: 5.53%, 12 h: 2.87%) and highest and lowest CBG were measured at 18:00 and 02:00, respectively. In simulations, the lowest cortisol exposure was observed after administration of hydrocortisone at 23:00-02:00, whereas the highest was observed at 15:00-18:00. The differences between the highest and lowest exposure were minor (≤12.2%), also regarding the free cortisol concentration and free fraction (≤11.7%).

Conclusions: CBG has a circadian rhythm but the difference in cortisol exposure is ≤12.2% between times of highest and lowest CBG concentrations; therefore hydrocortisone dose adjustment based on time of dosing to adjust for the CBG concentrations is unlikely to be of clinical benefit.

ClinicalTrials.gov registration number: NCT01960530

Key words: Hydrocortisone, Transcortin, Pharmacokinetics, Circadian Rhythm

Introduction

Oral hydrocortisone is the first-line glucocorticoid replacement therapy in patients suffering from adrenal insufficiency; recommended dosing in adults is 15-25 mg divided into two to three doses and in children 8 mg/m^2 into three-four doses per day(1). Replacement therapy aims to mimic the endogenous circadian rhythm of cortisol, which is challenging due to the relatively short half-life of hydrocortisone (1.5 h)(2). Hydrocortisone, therefore, needs to be administered several times a day to maintain adequate cortisol concentrations throughout the day. To determine an optimal dosing regimen it is important to understand the factors that influence hydrocortisone pharmacokinetics.

The absorption of hydrocortisone is fast and the maximum cortisol concentrations (C_{max}) are observed 1-1.4 h post-dose for 10-30 mg(2, 3) with approximately complete oral bioavailability(4). Absorption is delayed by food intake(5), and at higher doses(3). The area under the cortisol concentration-time curve (AUC) and C_{max} of hydrocortisone are dose-dependent after intravenous and oral administration, resulting in a less than proportional increase in exposure in relation to the dose(3, 6). This nonlinearity is partly related to the saturable binding of cortisol to the low capacity protein corticosteroid-binding globulin (CBG): if total cortisol concentrations approach maximum binding capacity of CBG (B_{max} ~ 500 nmol/L)(7, 8), there is an increase in unbound cortisol concentration (C_u). Since only unbound cortisol is distributed and eliminated, an increased unbound fraction leads to increased total cortisol clearance (CL) and volume of distribution (V). Rapid and distinct changes in CBG may therefore result

in changes in C_u, CL, V and, thereby, potentially the exposure (C_{max}, AUC) of hydrocortisone. Cortisol is also bound to albumin with a low affinity but high capacity. Rapid changes in albumin are therefore of less importance for cortisol pharmacokinetics.

CBG has a circadian rhythm and it has been hypothesised that this could impact hydrocortisone exposure depending on whether the dose is administered in the morning or in the evening(9, 10). A recent study based on CBG measurements during daytime (08:00-19:00) did not identify a circadian rhythm of CBG, probably since CBG concentrations were not assessed during the night time period. The authors therefore concluded that the potential impact of circadian CBG on cortisol exposure was not a concern(10). Other studies have identified a circadian rhythm of CBG, but did not aim to evaluate its potential impact on hydrocortisone exposure(11, 12). To evaluate whether hydrocortisone dosing should be adapted according to clock time, we used CBG concentrations over 24 h to develop a model describing the time course of CBG. The established CBG model was linked to a semi-mechanistic pharmacokinetic model for hydrocortisone to assess the impact on cortisol exposure (AUC and C_{max} of total cortisol) after dosing hydrocortisone at different clock times and of changing CBG concentrations.

Materials and Methods

CBG measurements

A total number of 350 CBG concentrations from a previously published study (ClinicalTrials.gov identifier: NCT01960530(12, 13)) with healthy volunteers were used for this analysis. The study was approved by the South East Wales Research Ethics committee, and performed according to local and international guidelines (ICH guideline for good clinical practice(14) and the Declaration of Helsinki(15)). Healthy volunteers in the age range of 18-60 years old with BMI between 21-28 kg/m^2, without known cardiac, liver or renal disease, were included in the study after giving written consent. Individuals working night shift, smoking, using regular medication or with respiratory, cardiovascular, metabolic, central nervous system or gastrointestinal tract dysfunction were excluded. 14 individuals

in the age of median (range) 28.5 years (22-60) with body weight of 82.9 kg (63.6-103) were included in the study, from whom CBG concentrations measured over 24 h (from 15:00 on day one to 15:00 on day two) in absence of treatment, were used for the current analysis. Participants slept between 23:00 to 06:00 with the lights out, received standardised meals at 13:00, 19:00 and 08:00, and were asked not to eat or drink 30 min before plasma sampling. CBG was sampled, diluted and quantified using an ELISA (Biovendor, Brno, Czech Republic) with a lower limit of quantification of 3.13 ng/mL and intra- and inter-assay variability <3% coefficient of variation (CV) and <8% CV, respectively(16). None of the CBG concentrations were below the lower limit of quantification.

Circadian CBG model

CBG concentrations from the study were analysed using a population approach in NONMEM 7.3(17). To best capture circadian processes, cosinor analysis was applied, which includes addition of one or several cosine functions(18): In this study, 1-3 cosine functions were added to describe the circadian rhythm of the CBG concentrations. Equations for two cosine functions with a periodicity of 24 h and 12 h (CIRC24, CIRC12) are exemplified in Eq. 1-2, in which the amplitude (AMP_{24} and AMP_{12}) and the time shift ($shift_{24}$ and $shift_{12}$) of the cosine functions are estimated. The respective circadian functions were sequentially added to the CBG baseline ($CBG_{baseline}$) in a proportional manner (Eq. 3). Variability between individuals was quantified using an exponential model, whereas a proportional model was used to consider variability within individuals (residual variability). Patient characteristics, such as body weight, height and age, explaining variability in the circadian CBG model were assessed. Visual predictive checks (VPC) were performed to assess how well the established model could predict the observed data: 1000 new datasets were simulated from the final model in NONMEM, from which the 5^{th}, 50^{th} and 95^{th} percentiles of the CBG concentrations were extracted and compared graphically with the corresponding percentiles from the measured CBG concentrations in R (VPC package(19))

$$CIRC_{24} = AMP_{24} \cdot \cos\left(\frac{2 \cdot \pi \cdot (time - shift_{24})}{24}\right) \quad \text{(Eq. 1)}$$

$$CIRC_{12} = AMP_{12} \cdot \cos\left(\frac{2 \cdot \pi \cdot (time - shift_{12})}{12}\right) \quad \text{(Eq. 2)}$$

$$CBG = CBG_{baseline} \cdot (1 + CIRC_{24} + CIRC_{12}) \quad \text{(Eq. 3)}$$

Simulations

By using a modelling and simulation approach, we aimed to systematically evaluate the impact on cortisol exposure (*AUC* and C_{max} of total cortisol) of changing CBG concentrations. In order to do so, the predicted CBG concentrations based on the above described CBG model was linked to a previously published semi-mechanistic pharmacokinetic (PK) model for hydrocortisone(13) (using a novel paediatric hydrocortisone formulation with taste masking). The PK model consisted of a two-compartment disposition model with saturable absorption (Michaelis-Menten absorption) and a plasma protein binding model. The binding model considered both the nonlinear binding to CBG – predicted by the circadian model – and linear binding to albumin or erythrocytes. More details regarding the semi-mechanistic PK model for hydrocortisone can be found in Melin *et al*(13).

The impact of changing CBG on simulated cortisol exposure was evaluated in a structured trial setting (scenario 1) and in a clinical use setting (scenario 2) for a typical patient (body weight was fixed to 70 kg).

Scenario 1: Impact of circadian CBG concentrations on cortisol exposure after single HC dose administration at different clock times (structured trial setting)

The simulations were done in a stepwise manner: first the established circadian CBG model was used to simulate individual CBG concentration-time profiles over 24 h in a virtual patient population (n=100), allowing for variability in CBG concentrations between individuals. In the second step, cortisol exposure (*AUC*, C_{max}) in the virtual population was simulated (allowing for variability in

cortisol PK parameters between individuals) after administration of single hydrocortisone doses (0.5, 2, 5, 10 or 20 mg) at every hour of the day in 120 different scenarios (= 5 doses at 24 different administration times) to assess impact of dosing hydrocortisone at different clock times. The lowest and highest *AUC* (AUC_{low}, AUC_{high}) and C_{max} ($C_{max,low}$, $C_{max,high}$) of the individual cortisol PK profiles for every dose level and every dosing time were identified and compared according to Eq. 4 and 5, to derive the % increase from the lowest to highest exposure (% difference *AUC* and % difference C_{max}).

$$\%\ difference AUC = 100 \cdot \frac{AUC_{high} - AUC_{low}}{AUC_{low}} \quad \text{(Eq. 4)}$$

$$\%\ difference\ C_{max} = 100 \cdot \frac{C_{max,high} - C_{max,low}}{C_{max,low}} \quad \text{(Eq. 5)}$$

Scenario 2: Impact of circadian CBG concentrations on cortisol exposure after a recommended dosing regimen (clinical use setting)

In scenario 2, cortisol concentration time-profiles were simulated using the established semi-mechanistic PK model(13) assuming either circadian (N=100) or constant (N=100) CBG profiles, respectively. *AUC* from dosing to 8 h post-dose ($AUC_{0\text{-}8h}$) and C_{max} for cortisol were derived in the population with circadian (AUC_{circ}, $C_{max,circ}$) and constant (AUC_{const}, $C_{max,const}$) CBG concentrations assuming a recommended thrice daily dosing (10 mg at 06:00, 5 mg at 14:00 and 5 mg at 22:00) for adults(1). The % difference in *AUC* and C_{max} by assuming circadian instead of constant CBG concentrations (% *difference* AUC_{circ} and % *difference* $C_{max,circ}$) were derived according to Eq. 6 and Eq. 7.

$$\%\ difference\ AUC_{circ} = 100 \cdot \frac{AUC_{circ} - AUC_{const}}{AUC_{const}} \quad \text{(Eq. 6)}$$

$$\%\ difference\ C_{max,circ} = 100 \cdot \frac{C_{max,circ} - C_{max,const}}{C_{max,const}} \quad \text{(Eq. 7)}$$

The simulations were done using NONMEM 7.3 (17), and the graphical evaluation and comparisons were done in R(20) for both scenarios.

Impact on free cortisol

Since only total cortisol concentrations were considered in the current work so far, the pharmacokinetic model(13) was used to predict the free, i.e. unbound, cortisol concentration and free cortisol fraction for total cortisol concentrations ranging to the upper confidence level for C_{max} after administration of 20 mg hydrocortisone (~900 nmol/L) for the minimum (median) and maximum (median) CBG concentrations observed over 24 h in the current population.

Results

Circadian CBG concentrations

CBG concentrations obtained over the 24 h showed a clear circadian rhythm (Fig. 1, left). The maximum CBG concentration (C_{maxCBG}, median (range)) was 24.3 (20.0-29.5) µg/mL, representing a 32.0% difference between the lowest and the highest C_{maxCBG}. The minimum CBG concentration (C_{minCBG}) was 20.4 (15.9-23.5) µg/mL, with a difference comparable to C_{maxCBG} (32.2%). The relative change in CBG concentrations during 24 h (C_{maxCBG}/C_{minCBG}) was 23.0% (16.4-38.8%), indicating that the variability within an individual is approximately equal to the variability between individuals. The time of C_{maxCBG} (t_{maxCBG}) and C_{minCBG} (t_{minCBG}) were (median (interquartile range)) 18:00 (with a smaller range of 18:00-19:00) and 03:30 (with a large range of 03:00-08:45), respectively.

Circadian CBG model

The circadian rhythm of CBG was well described with two cosine terms added to the baseline of CBG: The estimated baseline for CBG was 21.8 µg/mL and the associated interindividual variability was 11.9% CV (Table 1); the amplitude for the 24 and 12 h cosine functions were relatively small (24 h: 5.53%, 12 h: 2.87%). The predicted C_{maxCBG} (18:00) and C_{minCBG} (02:00) were in well agreement with the observed values. As seen in the VPC, comparing the percentiles of the observed data (black) and the simulated data (n=1000) using the final circadian CBG model (grey), the model could well predict the observed concentrations (Fig. 1, right). Addition of any covariates, such as height or body weight, to explain the variability in baseline was not supported by the data.

Impact on cortisol exposure

The impact of changing CBG on simulated cortisol exposure was assessed in a structured trial setting (scenario 1) and in a clinical use setting (scenario 2) as follows.

Scenario 1: Impact of circadian CBG concentrations on cortisol exposure after single HC dose administration at different clock times (structured trial setting)

In scenario 1, the impact of dosing time of HC across 24 h of the day on *AUC* and C_{max} for cortisol was simulated across all 5 doses (0.5-20 mg). The lowest and highest median *AUC* (AUC_{high}, AUC_{low}) and median C_{max} ($C_{max,low}$, $C_{max,high}$) for each different simulated dose levels are summarised in Table 2, and revealed that the maximum difference in *AUC* (*% difference AUC*) was relatively small (9.48%-12.2%), with the largest difference observed for the lower doses. The % difference C_{max} ranged from 4.20% to 9.01%. As seen in Fig. 2 (upper panels), the lowest cortisol exposure (AUC_{low}) was observed for doses administered between 23:00-01:00, whereas AUC_{high} was observed for doses administered in the afternoon (15:00-16:00). The lowest and highest C_{max} was observed for doses administered 01:00-02:00 and 17:00-18:00, respectively. These times were slightly delayed compared to times for lowest and highest *AUC*.

Scenario 2: Impact of circadian CBG concentrations on cortisol exposure after a recommended dosing regimen (clinical use setting)

In scenario 2, the impact of assuming circadian or constant CBG concentrations on cortisol exposure ($AUC_{0\text{-}8h}$, C_{max}) in the clinical setting was simulated using the semi-mechanistic PK model for hydrocortisone with the constant(13) or circadian CBG model. As seen in Fig. 2 (lower panels), the simulated $AUC_{0\text{-}8h}$ was slightly lower when assuming circadian CBG profiles (light grey, AUC_{circ}) compared to constant CBG profiles (dark gray, AUC_{const}) for the doses in the morning *(% difference* AUC_{circ}: -8.29%) and evening *(% difference* AUC_{circ}: -10.4%); the *% difference* AUC_{circ} for the

afternoon dose was low (-2.79%). The impact of considering the circadian rhythm of CBG was small for C_{max} for all doses, for which % difference $C_{max,circ}$ were ranging from -4.57% to -7.31% (Table 2).

Impact on free cortisol

The predicted free cortisol concentration and free fraction of cortisol for a large range of total cortisol concentrations are presented in Fig. 3. The free cortisol concentrations for the minimum (in black) and maximum (in grey) CBG were 105 and 92.6 nmol/L respectively for 900 nmol/L (Fig. 3, left), corresponding to the upper confidence level of total cortisol after administration of 20 mg hydrocortisone. The largest difference in free cortisol fraction for cortisol was observed for the higher concentrations, for which the impact of free cortisol fraction was minor (11.7% vs 10.3%, Fig 3, right), respectively.

Discussion

Therapy optimisation of hydrocortisone remains challenging due to complex pharmacokinetics and it has been suggested that cortisol pharmacokinetics is not constant during 24 h (chrono-pharmacokinetics), due to the circadian rhythm of CBG(9). We have undertaken a detailed analysis employing a modelling and simulation approach to evaluate the impact of circadian CBG on cortisol exposure (AUC and C_{max} of total cortisol) after administration of hydrocortisone. The results show that although there is a circadian rhythm in CBG, the amplitude is relatively small and has little impact on cortisol exposure. It is therefore likely to be of little clinical relevance with respect to hydrocortisone dosing.

Previous studies have identified a circadian rhythm of CBG(11, 21), whereas other studies have failed to do so(10). In the present study, the hourly CBG measurements during a 24 h period showed a clear circadian rhythm. In the current analysis, the circadian rhythm of CBG over a full day was well described by adding two cosine functions to the CBG baseline model. The precision of the parameters quantifying the circadian behaviour was good and both C_{maxCBG} and C_{minCBG} were well predicted by the

model. However, it should be noted that the observed CBG concentrations in our study were approximately constant during the daytime, which could explain why a constant CBG model was used to describe the data from 07:00-19:00(13) in the previous HC PK analysis. Since the CBG concentrations used in the current analysis were measured in healthy male volunteers, the impact of higher CBG concentrations in females compared to males(22) and in females on estrogen contraceptives (23, 24) or lower CBG concentrations in obese patients(22) could not be considered. However, higher CBG concentrations are expected to result in less fluctuation, and hence less impact on cortisol exposure than the scenario assessed in this analysis. Lower CBG concentrations may however result in larger fluctuations and more profound impact on cortisol exposure than observed in this analysis, which should be evaluated further in clinical studies.

In order to evaluate the potential impact of circadian CBG on cortisol exposure after administration of hydrocortisone, a previously established semi-mechanistic PK model of cortisol was combined with the here presented, validated circadian CBG model. First the impact of dosing time of HC administration was evaluated looking at the changes on cortisol exposure (AUC and C_{max}) when HC was administered at different hours. To do so, simulations were performed administering a single dose of a wide range of HC doses across the 24 possible hours of the day (structured trial setting). Secondly, the impact of considering the circadian CBG rhythm or not was compared for a clinically used dosing regimen (clinical setting). Even though CBG has a circadian rhythm, the prediction intervals for the lowest and highest simulated AUC and C_{max} of cortisol in scenario 1 were largely overlapping for all hydrocortisone doses including the high ones with nonlinear PK. The differences between the lowest and highest median AUC and C_{max} in the respective dose group were minor (*% difference AUC*: 9.48%-12.2%, *% difference* C_{max}: 4.20%-9.01%). These results indicate that the circadian rhythm of the binding protein CBG does not translate into a major difference in the exposure of cortisol. This could be due to the relatively small difference (~23%) between the lowest and highest CBG concentrations, which also resulted in relatively small amplitudes in the circadian variation.

The maximum CBG concentrations were measured around 18:00-19:00. During this time, the unbound fraction of cortisol is expected to be the lowest. Since maximum cortisol concentrations are observed approximately 1 h post-dose, the highest C_{max} for cortisol would be predicted for HC doses administered shortly before the observed time of maximum CBG concentration. This was in agreement with the $C_{max,high}$ of cortisol, which was observed for doses administered 17:00-18:00. The highest *AUC* of cortisol was observed for doses administered slightly earlier (15:00-16:00), probably due to the higher impact of clearance during the elimination phase which occurs later. The lowest C_{max} and *AUC* were observed for doses administered at 01:00-02:00 and 23:00-01:00, respectively. These time ranges occurred shortly before the lowest CBG concentrations around 03:30, when the unbound fraction, is at its highest.

Scenario 1 allowed us to systematically explore the impact of hydrocortisone dosing throughout the day, however, it is not reflecting the "real" clinical scenario. Given that CBG concentrations remain fairly constant during daytime, we evaluated the impact of including the circadian rhythm of CBG using a real clinical setting, in which hydrocortisone was dosed according to a clinically relevant thrice daily dosing regimen. Median exposure assuming circadian CBG was in general (morning, evening dosing) slightly lower compared to assuming constant CBG. Assuming circadian instead of a constant CBG concentrations resulted in the largest difference in *AUC* for the morning and the evening dose (*% difference* AUC_{circ}: 9%-11%) probably since the circadian CBG concentrations were slightly lower than the constant CBG during these times. The interquartile ranges and 95% prediction intervals for *AUC* and C_{max} were however overlapping as in scenario 1, indicating that the difference is not clinically relevant.

Since our analysis was based on total cortisol concentrations, rather than the free, i.e. unbound, cortisol concentrations, we used the pharmacokinetic model to demonstrate the impact of changing CBG on the free cortisol concentrations and free cortisol fraction. As discussed in the introduction and seen in Fig. 3, free unbound cortisol rises with higher concentrations of cortisol and the proportion of free cortisol increases in respect of total cortisol as the cortisol concentration exceeds the binding capacity of CBG. At CBG concentrations corresponding to the minimum (median: 20.4 µg/mL) and

maximum (median: 24.3 µg/mL) CBG, the variation in the free fraction at higher concentrations was small (11.7% vs 10.3%), and of little clinical relevance. The slightly higher free fraction will also translate into a slightly higher clearance of high doses of hydrocortisone. As stated previously, cortisol is also bound to albumin, which also has a circadian rhythm (12). The circadian variation was however minor (median peak:through ratio: 1.09), and not likely to impact cortisol pharmacokinetics due to the low affinity but high capacity binding to cortisol.

To evaluate the clinical relevance of the differences in exposure, one may hypothesise how a difference in AUC or C_{max} may translate into difference in pharmacodynamic effect. Assuming that the pharmacodynamic effect mediated by cortisol upon binding to the glucocorticoid-receptor is linear, this may indicate a maximum of ~10% difference in effect depending on timing of dose. A 10% difference is relatively small compared to the variability in PK parameters (~25%-30%CV)(13) and variability associated with other sources. The impact of the circadian CBG rhythm on hydrocortisone exposure is, therefore, unlikely to be clinically relevant, and dose adjustments based on when the dose is administered are probably not required.

Conflict of interest

JM, NH and ZPPG have nothing to declare. RJR & MJW are Directors of Diurnal Ltd and hold stock. CK report grants from an industry consortium (AbbVie Deutschland GmbH & Co. KG, Boehringer Ingelheim Pharma GmbH & Co. KG, Grünenthal GmbH, F. Hoffmann-La Roche Ltd, Merck KGaA and SANOFI) and grants from the Innovative Medicines Initiative-Joint Undertaking ("DDMoRe") outside the submitted work.

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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Tables

Table 1. Parameter estimates for the circadian corticosteroid-binding globulin (CBG) model developed on CBG concentrations observed during 24 h.

	Typical parameter estimate	95% CI
Fixed-effects		
$Baseline_{CBG}$ [µg/mL]	21.8	20.3, 23.3
Amp_{24} [%]	5.53	4.80, 6.20
$Shift_{24}$ [h]	1.77	1.33, 2.27
Amp_{12} [%]	2.87	2.21, 3.42
$Shift_{12}$ [h]	15.7	15.4, 16.0
Interindividual variability		
$\omega Baseline_{CBG}$ [CV%]	11.9	7.76, 14.0
Residual variability		
σprop [CV%]	3.90	3.46, 4.32

95% confidence interval (95% CI), amplitude for 24 h cosine function (Amp24), time shift for 24 h cosine function (Shift24), amplitude for 12 h cosine function (Amp12), time shift for 12 h cosine function (Shift12), variance of log-normally distributed interindividual variability (ω), variance or proportional residual variability (σprop).

Table 2. Impact of circadian corticosteroid-binding globulin (CBG) concentrations on simulated hydrocortisone exposure (n=100). Scenario 1: After single oral administration of hydrocortisone (0.5-20 mg) every hour of the day. The lowest and highest simulated area under cortisol concentration curve (AUC_{low} & AUC_{high}) and maximum cortisol concentration ($C_{max, low}$ & $C_{max, high}$). The percentage difference for AUC and C_{max}. Scenario 2: Simulated AUC and C_{max} assuming constant (AUC_{const}, $C_{max,const}$) or circadian CBG (AUC_{circ}, $C_{max,circ}$) after a three times daily dosing of hydrocortisone (10 mg at 06:00, 5 mg at 14:00 and 5 mg at 22:00). The difference in AUC and C_{max} between groups with constant and circadian profile (% difference AUC_{circ}, % difference $C_{max,circ}$).

Scenario 1 Dose	AUC_{low} [a]	AUC_{high} [a]	*% difference AUC*	$C_{max, low}$ [a]	$C_{max, high}$ [a]	*% difference* C_{max}
0.5 mg	164 (87.6, 311)	183 (97.8, 346)	11.6	95.9 (60.5, 156)	100 (62.8, 163)	4.20
2 mg	515 (303, 890)	577 (341, 989)	12.2	260 (174, 373)	279 (185, 405)	7.42
5 mg	962 (608, 1580)	1070 (678, 1730)	11.3	412 (288, 557)	449 (313, 602)	9.01
10 mg	1510 (991, 2410)	1650 (1100, 2600)	9.60	517 (376, 684)	561 (409, 734)	8.42
20 mg	2390 (1620, 3740)	2620 (1780, 3980)	9.48	605 (442, 818)	652 (479, 871)	7.93
Scenario 2 Dose	AUC_{const} [a]	AUC_{circ} [a]	*% difference* AUC_{circ}	$C_{max, const}$ [a]	$C_{max, circ}$ [a]	*% difference* $C_{max, circ}$
Morning (10 mg)	1660 (1020, 2460)	1530 (992, 2210)	-8.29	574 (395, 721)	532 (395, 676)	-7.31
Afternoon (5 mg)	1080 (641, 1680)	1050 (654, 1590)	-2.79	472 (312, 602)	451 (321, 567)	-4.57
Evening (5 mg)	1080 (639, 1660)	965 (605.0, 1450)	-10.4	471 (311, 598)	439 (313, 555)	-6.70

[a]Median (95% confidence interval)

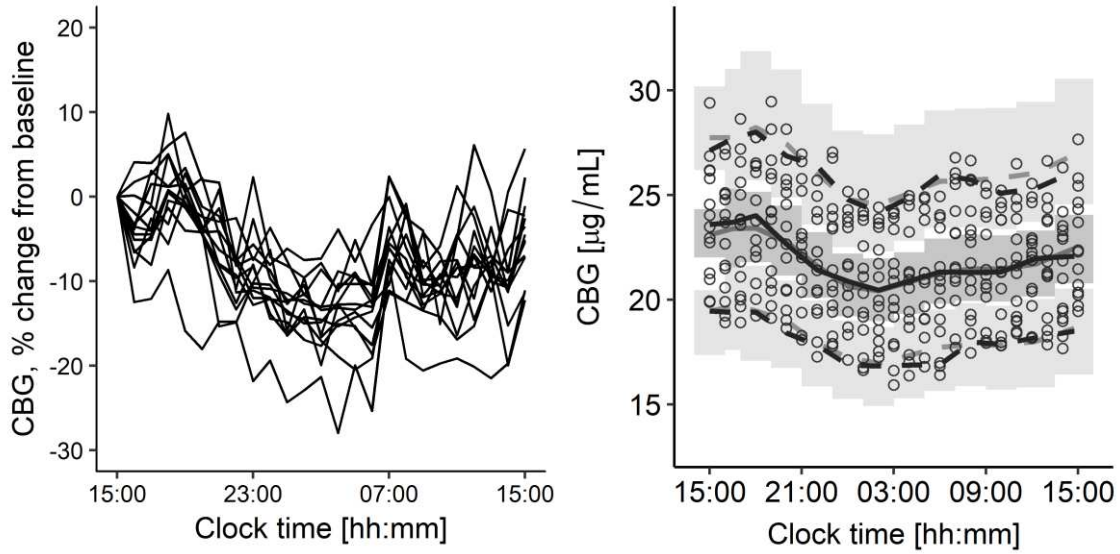
Figure legends

Figure 1 Left: Change in corticosteroid-binding globulin from baseline over time (left), during 24 h (n=14). Right: Visual predictive check for the circadian corticosteroid-binding globulin model during 24 h (15:00 day 1-15:00 day 2). Lines correspond to the 5th, 50th and 95th percentile of observed (black) and simulated (n=1000, grey) data. The areas are the 95th confidence interval around the percentiles and the circles the observations.

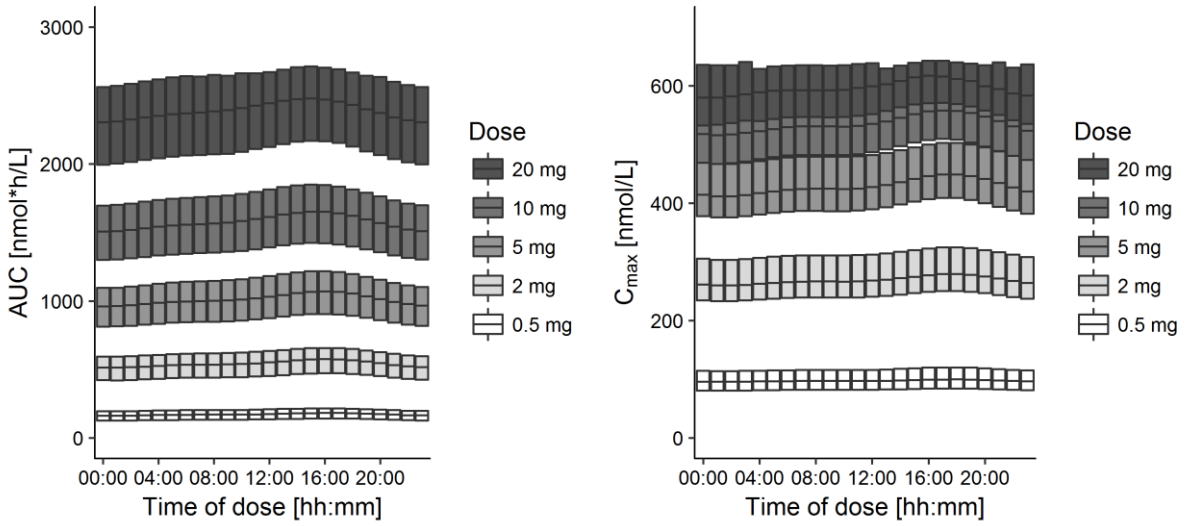
Figure 2 Impact on cortisol exposure: Simulation scenario 1 (top): Simulated area under cortisol concentration-time curve (*AUC*) and maximum cortisol concentration (C_{max}) after single oral administration of hydrocortisone every hour during 24 h to 100 individuals with different circadian corticosteroid-binding globulin (CBG) profiles. The box corresponds to the interquartile range; the line in the box the median.

Scenario 2 (bottom): Simulated AUC from dosing to 8 h post-dose ($AUC_{0\text{-}8h}$) and C_{max} after administration of hydrocortisone 10 mg in the morning (06:00), 5 mg in the afternoon (14:00) and 5 mg in the evening (22:00) for virtual patients with constant (dark gray, n=100) or circadian (light gray, n=100) CBG profiles, respectively. The box correspond to the interquartile range; the whiskers to the observations at most 1.5*interquartile range from the upper and lower quartiles; the line in the box to the median; the dots to outliers.

Figure 3 Predicted free, i.e. unbound, cortisol concentration versus total cortisol concentration (left) and predicted free cortisol fraction versus total cortisol concentration (right) for the maximum corticosteroid-binding globulin (CBG, 24.3 μg/mL) and minimum CBG (20.4 μg/mL) concentration based on the pharmacokinetic model(13)**.**



Scenario 1



Scenario 2