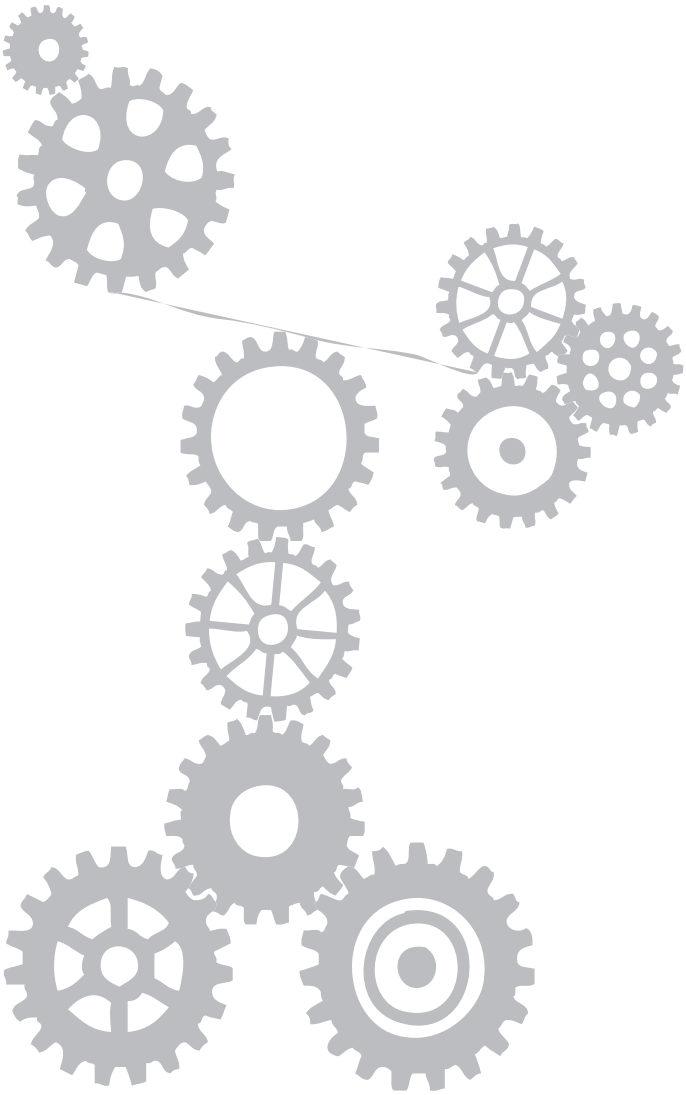
Chapter 7

Pharmacokinetics of hydrocortisone: results and implications from a randomized controlled trial



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ABSTRACT

Context and Objective

This study aimed at comparing pharmacokinetics of two different doses of hydrocortisone (HC) in patients with secondary adrenal insufficiency (SAI).

Design, Setting and Patients

Forty-six patients with SAI participated in this randomized double-blind crossover study.

Intervention

Patients received two different doses of HC (0.2–0.3 mg HC/kg body weight/day and 0.4–0.6 mg HC/kg body weight/day).

Main Outcome Measures

One- and two-compartment population models for plasma free cortisol, plasma total cortisol and salivary cortisol were parameterized. The individual pharmacokinetic parameters clearance (CL), volume of distribution (V_d), elimination half-life ($t_{1/2}$), maximum concentration (C_{max}), and area under the curve (AUC) were calculated.

Results

The one-compartment models gave a better description of the data compared to the two-compartment models. Weight-adjusted dosing reduced variability in cortisol exposure with comparable AUCs between weight groups. However, there was large inter-individual variation in CL and V_d of plasma free cortisol, plasma total cortisol and salivary cortisol. As a consequence, AUC_{24h} varied more than 10 fold. Cortisol exposure was increased with the higher dose, but this was dose proportional only for free cortisol concentrations and not for total cortisol.

Conclusions

Cortisol concentrations after a doubling of the dose were only dose proportional for free cortisol. HC pharmacokinetics can differ up to 10-fold inter-individually and individual adjustment of treatment doses may be necessary. Doubling of the HC dose in fast metabolizers (patients that showed relative low AUC and thus high clearance compared to other patients), does not result in significantly enhanced exposure during large parts of the day and these patients may need other management strategies.

INTRODUCTION

Hydrocortisone (HC) substitution by immediate release tablets is the most widely used glucocorticoid treatment to substitute for the lack of endogenous cortisol production in adrenal insufficiency (AI). Since the introduction of HC in the 50's, it is considered lifesaving, but it is also known for its imperfections with over- and undersubstitution during certain parts of the day.[1,2] To overcome this problem, several pharmaceutical products modifying the release of HC have been developed.[3,4] However, in spite of this progress, immediate release tablets remain the cornerstone of glucocorticoid substitution therapy today.

Various methods to assess the adequacy of glucocorticoid treatment regimen are suggested, but none of them is fully satisfactory. Some have advocated that clinical judgment is best to avoid over- or undersubstitution.[5,6] But considering that studies in patients with AI report increased mortality and morbidity rates in which undersubstitution as well as oversubstitution with glucocorticoids might play a role,[7] additional guidance is needed.

The use of biomarkers to assess the adequacy of HC replacement therapy has been promising, but it has not been successful so far.[8,9] Others use plasma cortisol day curves, but they are considered time-consuming, expensive and inconvenient for the patient,[10] and they lack sensitivity to discriminate under- and over-substituted patients from well-substituted patients.[5] Furthermore, total cortisol levels are usually measured, instead of free cortisol levels that is believed to reflect the biologically active form of cortisol. However, the measurement of plasma free cortisol is cumbersome and therefore not used in clinical practice. An alternative method to determine free cortisol concentrations is in 24-h urine samples, however, fluctuations in cortisol levels during the day are missed due to the nature of this method.[11] Assessment of cortisol in saliva has been reported to be a good alternative for free cortisol measurement as it is easy to perform.[12] However, there is large variation in the correlations between salivary cortisol and plasma total cortisol or plasma free cortisol, which may be induced by differences in glucocorticoids used or corticosteroid-binding globulin (CBG) concentrations.[11,13–15]

Alternatively, pharmacokinetic analyses have been performed.[1,11,16,17] This has led to recommendations for HC substitution therapy including thrice daily, weight related dosing, administered before the meal.[17] However, most of the studies performed either investigated healthy subjects,[16] had relatively small samples of patients,[11,17] or measured plasma total cortisol instead of plasma free cortisol.[1,11,16,17] Studies assessing the pharmacokinetics of hydrocortisone on free and total cortisol, both in plasma and saliva, and studying the effect of dose adjustments in patients with SAI have not been performed.

We have recently conducted a randomized double blind cross-over study aiming at assessing the effect of two different doses of HC on cognition (primary endpoint),[18] health related quality of life,[19] somatosensory functioning, and blood pressure.[20] In this ancillary analysis based on various cortisol measures we studied the pharmacokinetic parameters of two different doses of HC in patients with secondary AI with the aim to better understand individual and population pharmacokinetics and the effects of HC dose adjustments.

MATERIALS AND METHODS

Patients

Sixty-three patients were recruited between May 2012 and June 2013 from the endocrine outpatient clinic at the University Medical Center Groningen, the Netherlands. Sixty of these patients completed the run-in phase and the baseline measurement (mean [SD] age, 52 [13], range 19–73, 35 males) 47 of them completed both study periods. One more patient was excluded due to violation of the study protocol, therefore 46 patients were included in the present analysis. All patients were diagnosed with secondary AI according to internationally accepted criteria and had been on stable glucocorticoid replacement therapy for at least six months. Other potential pituitary hormone deficiencies were adequately substituted and remained stable for at least six months prior to entry of the study and during the study. Renal and liver function tests were normal in all patients. Patients using medication known to alter cytochrome P450 (CYP3A4) activity (e.g. anti-epileptics) were excluded. Other inclusion and exclusion criteria have been described elsewhere.[18]

The study protocol was approved by the local ethics committee at the University Medical Center Groningen, The Netherlands. Patients gave written informed consent before entering the study. This study is registered with ClinicalTrial.gov, number NCT01546922.

Intervention

Patients were randomly allocated to receive first a lower dose of 0.2–0.3 mg HC/kg body weight/day (total daily doses ranging from 15–20 mg HC depending on body weight) for ten weeks followed by a higher dose of 0.4–0.6 mg HC/kg body weight/day (total daily doses ranging from 30–40 mg HC depending on body weight) for ten weeks (group 1) or in reversed order (group 2). HC substitution was given in three divided doses (before breakfast, before lunch, before dinner) with the highest dose given in the morning. The exact dosing scheme has been published previously.[18] In case of intercurrent illness, patients were allowed to double or triple their dose, but not

for longer than 7 consecutive days. Patients were (strictly) instructed not to double or triple their dose in the week preceding the visit day.

Protocol

After each 10 week treatment period patients attended the hospital outpatient clinic. On the visit days, patients were instructed to take their morning dose of HC at 0700 h. At 0800 h they attended to the hospital and blood samples were drawn in sitting position after a short period of rest for the measurement of plasma total cortisol and plasma free cortisol, which was repeated approximately five hours later. On the day preceding the hospital visit, patients collected 24-h urine for the measurement of 24-h urinary free cortisol and urinary free cortisone. In addition, seven patients collected saliva samples using a Salivette (Sarstedt, Nümbrecht, Germany) at 25 moments on one day, starting on the day preceding the scheduled hospital visit for the measurement of salivary cortisol.

Laboratory measurements

Plasma free cortisol and plasma total cortisol, urinary free cortisol, urinary free cortisone as well as salivary cortisol were all measured by isotope dilution liquid chromatography tandem mass spectrometry (LC-MS/MS). Total and free cortisol in plasma were performed essentially as described by Hawley et al., using cortisol-$13C_3$ as internal standard.[21] For plasma free cortisol, intra- and interassay coefficients of variability (CVs) were less than 6%. For plasma total cortisol intra- and interassay CVs were less than 2.6%. Plasma equilibrium dialysis for free cortisol was performed as described by Fiers et al.,[22] with the only difference that we used 10 kD cellulose membranes (Harvard Apparatus; Holliston). Urinary free cortisol, urinary free cortisone and salivary cortisol assays were performed essentially as described by Jones et al.[23] Intra-assay CV for salivary cortisol were less than 11.3%. Serum CBG concentrations were determined in duplicate using a radioimmunoassay (IBL International GmbH, Hamburg, Germany). Albumin was measured using the brome cresol green method on a Roche Modular ISE/P (Roche Diagnostics, Mannheim, Germany).

Population pharmacokinetic analysis

Using the Kinpop module of the pharmacokinetic software package MwPharm version 3.81 (Mediware, Zuidhorn, The Netherlands), a one-compartment and a two-compartment population model were parameterized for plasma free cortisol, plasma total cortisol and salivary cortisol taking the assay error into account. One- and two-compartment models were parameterized using an iterative two-stage Bayesian procedure and medians, means and standard deviations (SD) of the pharmacokinetic parameters total body clearance (CL), intercompartment clearance (CL_{12}), apparent

volume of distribution of the central compartment (V_1) and apparent volume of distribution of the peripheral compartment (V_2) were estimated. The elimination half-life ($t_{1/2}$) was calculated from 0.693/*k*e, where *k*e is the elimination rate constant. The 'a priori' models with fixed estimates for the absorption constant of 1.4 h^{-1} and bioavailability of 96% based on existing literature were constructed.[24] The 'a priori' model was fit to the data to construct the final models. The pharmacokinetic parameters for plasma free cortisol at the lower HC dose and the higher HC dose were compared. If there was no difference, the final one-and two-compartment models were constructed with combined data from both doses.

Each model was validated with the Monte Carlo analysis using MwPharm. In this analysis, for all measures of cortisol, 100 patients were simulated per patient for the one-compartment and two-compartment models. Patient parameters were randomly calculated based on the estimated population parameter values. The relative root mean squared error (% RMSE), as a measure of precision of the one- and two-compartment models, was calculated. A RMSE below 15% was considered to be sufficient.[25] The model with the lowest RMSE was chosen for further pharmacokinetic analysis.

Individual pharmacokinetic parameters were calculated by maximum a posteriori Bayesian estimation using the one-compartment final model for plasma free cortisol, plasma total cortisol and salivary cortisol for the two different doses of HC. CL, volume of distribution (V_d), $t_{1/2}$, maximum concentration (C_{max}) and area under the curve (AUC) after the first, second and third dose (C_{max1}, AUC_1, C_{max2}, AUC_2, C_{max3} and AUC_3 for the first, second and third dose respectively) were calculated. AUC_{24h} was determined as the sum of AUC_1, AUC_2 and AUC_3.

Three patients had cortisol levels that were higher 5 hours after intake of the hydrocortisone than after 1 hour. Therefore, these subjects, representing about 6% of the study population, were not included in the population pharmacokinetic model. Other curves showing possible outliers after visual inspection were included, since population pharmacokinetic modeling and individual maximum a posteriori Bayesian estimation by iterative two-stage Bayesian analysis is robust for relatively small errors in registration.[26] For the model for salivary cortisol no outliers were removed, as they did not influence the model parameter estimates.

Statistics

The present analysis is a post-hoc pharmacokinetic analysis of a pharmacodynamic study.[18] The power analysis performed for the study was based on the primary endpoint of the pharmacodynamic study: cognitive performance after the two different HC doses.[18] A study arm with each 25 patients (50 patients in total) would be able to detect an effect size of 0.4 with $\alpha = 0.05$ and $\beta = 0.80$. In order to allow a dropout rate of approximately 20%, a total of 60 patients had to be included, as described previ-

ously.[18] Data of all patients that completed the pharmacodynamic study were used for pharmacokinetic analysis. Data are presented as mean (SD) or median (interquartile range). Normality of the data was checked using QQ-plots and histograms. Because of skewness of the data, non-parametric tests were used. Individual pharmacokinetic parameters for plasma free cortisol, plasma total cortisol and salivary cortisol on the two different doses of HC were compared by using the Wilcoxon Signed Rank Test for paired observations. Individual pharmacokinetic parameters were compared between three weight groups by using the Kruskal-Wallis Test with post hoc pairwise comparisons. Correlations were calculated using Spearman's correlation coefficient. All statistical analyses were performed using the Statistical Package for the Social Sciences version 22 (SPSS Inc., Armonk, N.Y., USA).

Table 1. Cortisol levels, CBG, albumin and glucocorticoid metabolites in patients with secondary adrenal insufficiency treated with two different doses of hydrocortisone (n = 46)

	Lower dose	Higher dose	*P*-value
Plasma total cortisol levels			
1 hr after ingestion (nmol/L)	495 [385; 598]	737 [671; 870][a]	< 0.001
5 hrs after ingestion (nmol/L)	112 [74; 195]	231 [170; 321]	< 0.001
Plasma free cortisol levels			
1 hr after ingestion (nmol/L)	30 [23; 44][a]	70 [49; 80][c]	< 0.001
5 hrs after ingestion (nmol/L)	4 [2; 8][b]	9 [6; 15][d]	0.003
Serum CBG (µg/ml)	52.8 [49.1; 61.9][a]	55.7 [48.1; 61.9][a]	0.193
Serum albumin (g/L)	46 [45; 49]	48 [45; 50][a]	0.010
Urinary free cortisol (nmol/24h)	78 [46; 112][a]	281 [200; 411][e]	< 0.001
Urinary free cortisone (nmol/24h)	229 [147; 374][a]	445 [326; 663][e]	< 0.001

Abbreviations: CBG: corticosteroid binding globulin.
Data are median [interquartile range].
P-value lower dose versus higher dose by Wilcoxon Signed Rank Test for paired observations.
[a] n = 45, [b] n = 38, [c] n = 43, [d] n = 37, [e] n = 44

RESULTS

Patient sample

Forty-six patients completed both study periods and were used in the present analysis (28 males, age 51 (14) years, range 19–73). The clinical characteristics of the study population have been published previously,[18] a summary is added as Supplemental Table 1.

Table 2. Population pharmacokinetic parameters and Monte Carlo Simulation of the lower and the higher dose combined

	Population estimates			Monte Carlo Simulation		
	Median	**Mean**	**SD**	**Mean**	**95% CI**	**RMSE (%)**
Plasma free cortisol (n = 89)						
CL (L/h)	244.13	235.78	108.22	236.22	217.83–254.61	2.5
V_d (L)	452.62	474.38	257.13	487.82	445.51–530.14	4.2
$t_{1/2}$ (h)	1.28	1.39	1.65	1.43	1.42–1.44	
Plasma total cortisol (n = 91)						
CL (L/h)	14.04	12.85	6.50	13.75	12.42–15.08	0.4
V_d (L)	41.75	39.82	14.85	39.97	37.24–42.70	0.5
$t_{1/2}$ (h)	2.06	2.15	1.58	2.02	1.96–2.08	
Salivary cortisol (n = 14)						
CL (L/h)	337.58	352.92	195.00	351.79	284.94–418.64	1.1
V_d (L)	486.25	348.37	445.02	447.35	229.21–665.50	10.4
$t_{1/2}$ (h)	1.00	0.68	1.58	0.83	0.56–1.10	

Abbreviations: CI: confidence interval; CL: total body clearance; n: number of curves included in the model; RMSE: Root Mean Squared Error; SD: standard deviation;
$t_{1/2}$: elimination half-life; V_d: volume of distribution.
The 'a priori' models were constructed with an absorption constant of 1.4 h^{-1} and bioavailability of 96%

Cortisol, CBG and albumin

The higher dose of HC resulted in significantly higher plasma total cortisol and plasma free cortisol concentrations (both 1 h and 5 h after ingestion of the morning dose), higher albumin, urinary free cortisol and urinary free cortisone concentrations compared to the lower dose of HC (Table 1). CBG concentrations did not change on the higher HC dose.

Population pharmacokinetics

The population parameter estimates did not differ between the lower dose and the higher dose, therefore the data of both doses were combined to parameterize the final one- and two-compartment population models. Population pharmacokinetic estimates for plasma free cortisol, plasma total cortisol and salivary cortisol are given in Table 2. The one-compartment models gave a better description of the data than the two-compartment models as indicated by the lower RMSE (< 15%) and thus were kept as the final models for the estimation of the individual parameters. There was considerable inter-individual variation in CL and V_d for the models of plasma total and free cortisol as well as salivary cortisol (Table 2 and 3, Figure 1). This resulted in a high variability in cortisol concentration-time curves. In Figure 1 a simulation of the concentration-time curve (with 95% confidence interval) is shown for plasma

total cortisol for a standard patient (weight of 70 kg, length of 1.75 m) based on the population pharmacokinetic parameters, for both the lower dose and the higher dose, illustrating the large inter-individual variation.

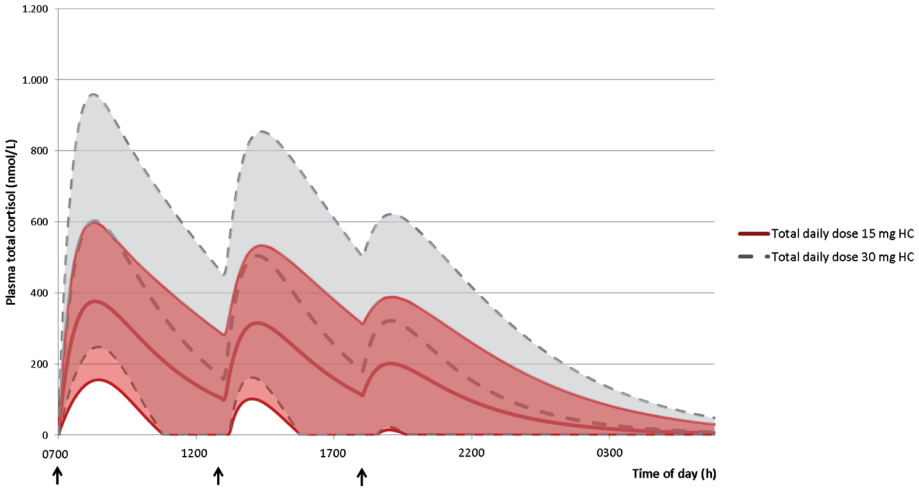

Figure 1. Concentration time profile of a standard patient (weight of 70 kg, length of 1.75 m). Median (thick solid and dashed line) with 95% CI (thin solid and dashed lines). The 95% CI is based on the variability of the model parameters and the variability of the biochemical assays used. Arrows indicate time points of hydrocortisone administration.

Pharmacokinetic values on the two doses of hydrocortisone

CL and V_d were comparable between the two doses of HC for plasma free cortisol and salivary cortisol (each $P > 0.05$) (Table 3). CL and V_d for plasma total cortisol were significantly higher after receiving the higher dose of HC compared to the lower dose ($P < 0.01$). Furthermore, $t_{1/2}$ was comparable between the lower and the higher dose for plasma free cortisol, and plasma total cortisol as well as for salivary cortisol. C_{max} for plasma total cortisol and plasma free cortisol was significantly higher after the higher dose (each $P < 0.001$), but this was only dose proportional for free cortisol concentrations and not for total cortisol. Thus, doubling of the HC dose resulted in 2.1 ± 0.9 the C_{max1} for plasma free cortisol and 1.5 ± 0.5 for plasma total cortisol ($P < 0.001$). The AUC_{24h} for plasma free cortisol was 2.2 ± 1.0 higher after doubling of the dose while this was 1.6 ± 0.6 for plasma total cortisol ($P < 0.001$). Large inter-individual variation was found, as AUC_{24hr} for plasma free cortisol and plasma total cortisol on the same dose varied by a factor of approximately 10 (Figure 2A and B).

To test whether age had an effect on our results, an additional correlation analysis was performed. For plasma free cortisol and plasma total cortisol, no pharmacokinetic parameter was associated with age, except for Vd of plasma total cortisol on the lower dose ($r = 0.358$, $P = 0.016$).

Table 3. Pharmacokinetic parameters for plasma free cortisol, plasma total cortisol and salivary cortisol for the two doses of hydrocortisone

	Plasma free cortisol		Plasma total cortisol		Salivary cortisol	
	Lower dose	**Higher dose**	**Lower dose**	**Higher dose**	**Lower dose**	**Higher dose**
CL (L/h)	226.21	227.11	12.46	15.27**	389.61	247.81
	[168.20; 336.90]	[178.83; 311.93]	[8.80; 17.38]	[11.93; 18.47]	[237.81; 643.26]	[177.33; 440.41]
V_d (L)	422.30	466.12	32.98	50.33***	619.07	339.62
	[315.88; 654.73]	[348.16; 607.55]	[26.01; 47.23]	[35.17; 58.76]	[136.18; 674.11]	[155.25; 611.90]
$t_{1/2}$ (h)	1.17	1.15	1.82	2.00	0.91	0.89
	[0.99; 1.73]	[0.98; 1.64]	[1.40; 2.82]	[1.78; 2.60]	[0.20; 1.79]	[0.56; 1.72]
C_{max1} (nmol/L)	32.69	70.81***	514.47	754.94***	28.53	96.71
	[25.13; 46.39]	[55.87; 80.78]	[390.28; 597.23]	[683.63; 903.35]	[17.91; 47.79]	[52.75; 119.76]
AUC_1	90.63	199.11***	1743.93	2533.02***	65.05	152.80*
(h*nmol/L)	[68.34; 119.66]	[147.30; 247.25]	[1246.04; 2133.44]	[2173.09; 3206.01]	[31.09; 81.57]	[123.37; 259.25]
AUC_{24h}	211.81	419.99***	3868.31	6433.07***	133.45	369.00*
(h*nmol/L)	[144.69; 260.02]	[324.58; 515.76]	[2834.99; 5349.00]	[5260.17; 7786.22]	[63.77; 165.88]	[289.32; 525.39]

Abbreviations: CL: total body clearance, V_d: volume of distribution, C_{max1}: maximal concentration after the first dose of hydrocortisone, AUC_1: area under the curve after the first dose of hydrocortisone, AUC_{24h}: 24-hour area under the curve.

Data are given as median [interquartile range].

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for lower dose vs higher dose by Wilcoxon Signed Rank Test for paired observations.

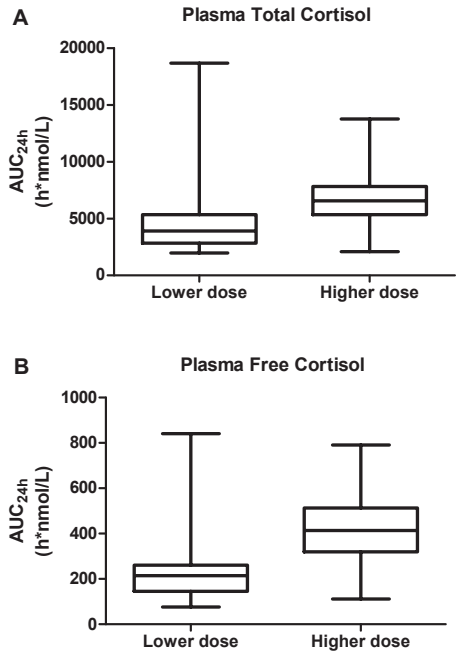

Figure 2. AUC_{24h}'s for plasma total cortisol (A) and plasma free cortisol (B) on the two doses of HC.

Weight-related dosing

AUCs and C_{max} of plasma free cortisol and plasma total cortisol for the three daily dose administrations did not differ between the three weight groups on the higher dose (Supplemental Tables 2 and 3). The same was true for the lower dose, with the exception of a minor difference in cortisol exposure after the third gift in which the lowest weight group had higher C_{max3} and AUC_3 compared to the highest weight group, both for plasma free cortisol and plasma total cortisol.

Associations between the various measures of cortisol

CL, V_d and AUC_{24h} of free cortisol at the lower dose of HC correlated significantly with CL, V_d and AUC_{24h} of free cortisol at the higher dose ($r = 0.476$, $r = 0.467$, $r = 0.449$ for CL, V_d and AUC_{24h} respectively, each $P < 0.003$). Similar but stronger correlations were found for total cortisol ($r = 0.655$, $r = 0.540$, and $r = 0.634$ for CL, V_d and AUC_{24h} respectively, each $P < 0.001$).

AUC_{24h} for plasma free cortisol correlated significantly with AUC_{24h} for plasma total cortisol at the lower dose of HC ($r = 0.872$, $P < 0.001$) as well as at the higher dose ($r = 0.752$, $P < 0.001$, Figure 3A). Urinary free cortisol correlated significantly with AUC_{24h} for plasma free cortisol (lower dose: $r = 0.563$, $P < 0.001$; higher dose:

Table 4. Overview of literature assessing pharmacokinetic properties of hydrocortisone.

Study	Design	N	Age (year)	Body weight (kg)	Outcome measures
Derendorf et al., 1991	Intervention study	8 healthy male subjects	28 ± 9	74 ± 6	Pharmacokinetic parameters were estimated for total cortisol using compartmental and non-compartmental analysis.
Mah et al., 2004	Intervention study	6 patients with PAI 4 patients with SAI	46–68 (range)	56.6–112.4 (range)	Pharmacokinetic parameters were estimated for total cortisol with a user-defined model with *P*-Pharm software.
Thomson et al., 2007	Intervention crossover study	9 patients with PAI 18 patients with SAI	47 ± 10	75 ± 14	Noncompartmental individual pharmacokinetic parameters were determined using WinNonlin.
Simon et al., 2010	Prospective, open-label study	20 patients with PAI 30 patients with SAI	53 ± 17	74 ± 18	Population pharmacokinetic modelling for total cortisol was done by NONMEN. One and two-compartment models were calculated.
Present study	Intervention study (RCT double-blind crossover)	46 patients with SAI	51 ± 15	83 ± 14	Population pharmacokinetic models were calculated for plasma free cortisol, plasma total cortisol and salivary cortisol.

CL = clearance, V_d = volume of distribution, $t_{1/2}$ = elimination half-life, HD = hydrocortisone, IV = intravenous, PAI = primary adrenal insufficiency, SAI = secondary adrenal insufficiency. RCT = randomized controlled trial.
Data are presented as mean ± SD or median (interquartile range), unless otherwise stated.

Intervention	Results		
	CL (L/h)	V_d (L)	$t_{1/2}$ (h)
Endogenous cortisol production was suppressed by the administration of 4 mg dexamethasone at 2200 h the day preceding the study. 20 mg of HC was administered IV.	18.2 ± 4.2 (L/h)	33.7 ± 4.6 (L)	1.82 ± 0.52 (h)
Patients received 10 mg HC orally.	16.2 ± 1.62 (L/h)	NA	NA
On day 1 patients received 20 mg HC IV. On day 2 patients received their own oral HC dose (10, 15 or 20 mg HC).	Plasma IV: 20.2 (16.3–23.1) (L/h) Plasma oral: 19.1 (14.4–21.9) (L/h) Saliva IV: 256 (194–376) (L/h) Saliva oral: 215 (181–292) (L/h)	Plasma IV: 61.9 (51.0–72.7) (L) Plasma oral: 52.3 (40.6–57.4) (L) Saliva IV: 608 (338–1138) (L) Saliva oral: 457 (331–774) (L)	Plasma IV: 2.16 (1.94–2.40) (h) Plasma oral: 1.81 (1.69–2.36) (h) Saliva IV: 1.74 (1.01–2.37) (h) Saliva oral: 1.47 (1.24–1.72) (h)
PAI patients received 28 mg (median 30 [range 20–50] mg) HC (0.41 mg/kg body weight). SAI patients received 23 mg (median 20 [range 15–30] mg) HC (0.32 mg/kg body weight).	12.1 (SE (%) 6.1) (L/h)	38.7 (SE (%) 8.30) (L)	1.7 (95% CI 1.5–1.9) (h)
Patients received a lower dose for 10 weeks (0.2–0.3 mg/kg body weight) followed by a higher dose for 10 weeks (0.4–0.6 mg/kg/body weight). Or in reversed order.	Plasma total cortisol: 12.85 ± 6.50 (L/h) Plasma free cortisol: 235.78 ± 108.22 (L/h) Saliva cortisol: 352.92 ± 195.00 (L/h)	Plasma total cortisol: 39.82 ± 14.85 (L) Plasma free cortisol: 474.38 ± 257.13 (L) Saliva cortisol: 348.37 ± 445.02 (L)	Plasma total cortisol: 2.15 ± 1.58 (h) Plasma free cortisol: 1.39 ± 1.65 (h) Saliva cortisol: 0.68 ± 1.58 (h)

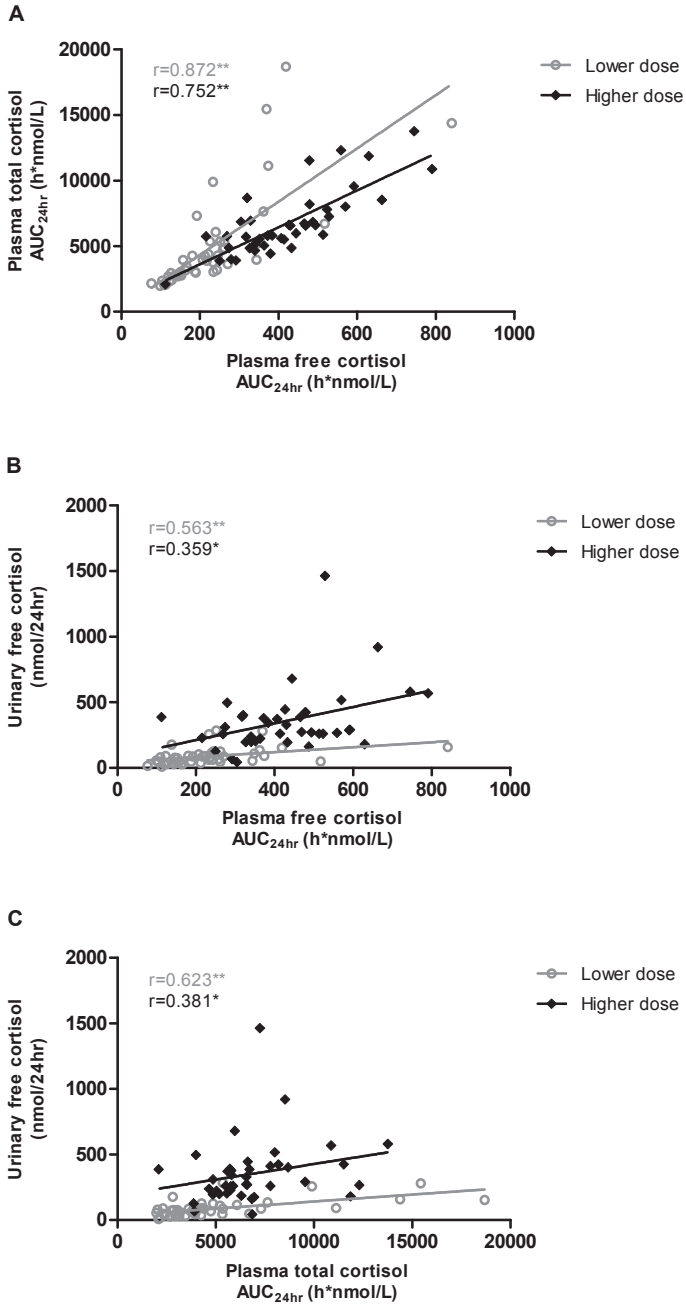

Figure 3. Correlation of plasma free cortisol AUC_{24h} with plasma total cortisol AUC_{24h} (A), plasma free cortisol AUC_{24h} with urinary free cortisol (B), and plasma total cortisol AUC_{24h} with urinary free cortisol (C).

* $P < 0.05$, ** $P < 0001$

$r = 0.359$, $P < 0.05$, Figure 3B) and with AUC_{24h} for plasma total cortisol (lower dose: $r = 0.623$, $P < 0.001$; higher dose: $r = 0.381$, $P < 0.05$, Figure 3C).

DISCUSSION

Our results show that HC pharmacokinetics can differ up to 10-fold between individuals. A doubling of the HC dose results only in a doubling of the plasma free cortisol concentrations, whereas the increase in total cortisol concentrations was lower. Furthermore, in patients that showed relatively high CL and low AUC compared to other patients in the present study, designated as fast metabolizers, a doubling of the dose does not result in markedly enhanced cortisol exposure during large parts of the day. Based on pharmacokinetic characteristics, HC dosing should therefore be individualized in case of maintenance therapy. Attention should be paid to stress-dosing since doubling of the dose does not necessarily result in doubled exposure, and fast-metabolizers may not benefit from it in terms of marked increments of plasma cortisol concentrations.

This is the largest study to date on pharmacokinetics of immediate release HC. Our extensive laboratory determinations together with pharmacokinetic analysis provide data on free and total cortisol in both plasma and saliva under two HC dose conditions. In addition, other measures of HC metabolism like urinary free cortisol, CBG and albumin were taken into account. Another strength of the present study is that pharmacokinetic variables were calculated based on plasma cortisol concentrations measured with the LC-MS/MS, instead of previously used immunoassays, providing greater analytical specificity.[27]

A few limitations of the study need to be addressed. First, our population pharmacokinetic model was based on cortisol concentration measurements of two time-points, whereas most pharmacokinetic studies are based on elaborate regularly timed cortisol measurements. However, the Kinpop module of MwPharm using the Bayesian procedure is developed specifically for parameterizing population pharmacokinetic models based on scarce data from a relatively large patient sample, thereby circumventing this problem. In addition, the pharmacokinetic outcome variables were comparable to other studies (Table 4), and even related to urinary free cortisol concentrations. In agreement, a study by Rousseau and colleagues showed that single point cortisol concentrations correlated significantly with cortisol $AUC_{0800–1900hr}$ in patients with primary AI.[28] Thus, even without extensive individual sampling we could reliably perform a pharmacokinetic analysis. Second, although we tried to standardize the salivary cortisol collection by using protocols, sampling was done at home without supervision of the investigators. In addition, data on salivary cortisol were performed in a subset of patients and not in the entire study population, which could have led to missing a true effect due to

the lack of statistical power. In future studies, it would be preferable to collect salivary cortisol in a larger patient sample along with blood sampling at the same time points. Third, CBG binding capacity varies dependent on the cortisol concentrations and this variation is known to influence pharmacokinetic parameters of cortisol.[14] MwPharm is unable to take this variation in protein binding capacity into account and calculate its effect on CL. Consequently, linear models with a relatively higher variance are parameterized for total cortisol instead of non-linear models. Future studies should use a program incorporating this variability in protein binding.

In agreement with other studies, we found large inter-individual differences in pharmacokinetic parameters.[1,11] Several factors can influence the variability in pharmacokinetic values, for instance variation in CYP3A4 enzyme activity. CYP3A4 is an important enzyme in the metabolism of cortisol and its activity can vary 5 to 20-fold inter-individually.[29] CYP3A4 activity can be induced by drugs like rifampicin and several anticonvulsant agents, and inhibited by drugs such as azole antifungal agents and macrolide antibiotics. We did not measure CYP3A4 activity nor determined CYP3A4 genotypes, however, we excluded patients using these enzyme inducers or inhibitors. We also corrected for body weight, which is known to influence cortisol clearance.[17] Indeed, the administration of weight-adjusted doses resulted in highly comparable cortisol exposure between weight groups. However, on an individual basis variability in cortisol exposure was still substantial, indicating the need for individual dose adjustments. Approximately 6% of our patients had higher cortisol levels 5 hours after administration compared to 1 hour after administration. These patients may have had very slow resorption of cortisol and/or very slow elimination. Residual cortisol production to explain this seems very unlikely since morning (0800 h) cortisol concentration are invariably higher than later during day time.

Based on population pharmacokinetics, a substantial percentage of patients is predicted to have plasma total cortisol levels well below 100 nmol/L during large parts of the day (Figure 1). Although the functioning of the hypothalamic-pituitary-adrenal axis is not only represented by cortisol concentrations in plasma, in patients this plasma concentration is frequently used as a cut-off level below which adrenal insufficiency is diagnosed. Thus, when complaints compatible with undersubstitution are present and they are accompanied by low plasma cortisol concentrations, it appears reasonable to raise cortisol concentrations by dose adjustments. However, one must bear in mind that other determinants of the cortisol metabolism, like 11β-hydroxysteroid dehydrogenase activity, cortisone concentrations, intracellular concentrations, and the effects of cortisol efflux transporters, must be considered.

Another point of attention is that doubling of the dose does not necessarily increase cortisol concentrations by a factor of 2. This is also found in previous studies, showing a non-proportional increase in cortisol exposure after increasing doses, both after

administration of immediate release tablets [30,31] and after administration of dual-release HC tablets.[3,32] However, different results are found when looking at free cortisol. Our results show that for plasma free cortisol AUC_{24h} values were dose proportional to the HC dose administered. For AUC_{24h} values for salivary cortisol this was even higher, as they increased by factor 3 on the higher dose. Whether this difference in behavior in response to dose increments is relevant remains elusive. The free fraction of cortisol is expected to represent the biologically active part of cortisol, but CBG, that binds approximately 80% of cortisol, is not only known as an inactive carriers, but also as a protein that delivers cortisol when its binding capacity is decreased upon cleaving of the reactive center loop by proteases,[33] assisting in tissue specific delivery of cortisol. It could be surmised that for patients with persistent complaints compatible with undersubstitution, not an increase in total daily dose but an increase in the number of daily doses or the administration of modified release HC would be beneficial if fast metabolism of cortisol is a contributing mechanism.

The non-proportional total cortisol exposure after a doubling of the dose may be a consequence of increased clearance of cortisol due to saturation of CBG. Saturation of CBG occurs at total plasma cortisol concentrations of 450–550 nmol/L,[34,35] and consequently, the higher dose of oral HC used readily saturates CBG, leading to increases in free plasma cortisol, salivary cortisol and urinary cortisol.[13] This is accompanied by a significant increase in CL of plasma total cortisol (Table 3) to restore the balance resulting in a relatively decreased total cortisol exposure.

It is known that aging may have pharmacokinetic implications[36]. Aging is associated with reduced fist-pass metabolism, increasing bioavailability of a few drugs. With advancing age body fat increases and total body water and lean body mass decreases, leading to an increased volume of distribution and prolonged half-life for lipophilic drugs such as cortisol. Furthermore, a reduction in renal and hepatic clearance is found with advancing age. In our study with patients aged between 19–73 year we found minor evidence that this influenced our results. Only total cortisol V_d on the lower dose was positively associated with age, while other pharmacokinetic indices on both different doses remained non-significant.

In conclusion, pharmacokinetics of HC can differ 10-fold between individuals. A doubling of the dose does not result in a doubling of total cortisol exposure. Furthermore, fast-metabolizers may not benefit from dose increments with regard to improved concentrations-time curves, and other managing strategies should be considered. Due to the large inter-individual variation individually tailored treatment doses and regimens are required.

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SUPPLEMENTAL DATA

Supplemental Table 1. Clinical characteristics at baseline of pituitary patients treated for secondary adrenal insufficiency (N = 46)

Age (y), median [IQR]	54 [42; 61]
Sex (males/females), n	28/18
Educational level (1/2/3/4/5/6/7) a	0/2/1/6/20/15/2
Body weight (kg), median [IQR]	84.0 [73.4; 93.1]
Hydrocortisone treatment prior to randomization	
Total daily dose (mg/day), median [IQR]	25 [20; 30]
Total dose/kg body weight/day (mg/kg/day), median [IQR]	0.32 [0.25; 0.35]
Number of daily dosings (1/2/3), n	3/33/10
Duration of hydrocortisone treatment (y), median [IQR]	12 [5; 23]
No. of hormonal replacements (1/2/3/4/5)	3/9/20/11/3
Thyroid hormone deficiency (% of patients)	91
Growth hormone deficiency (% of patients)	67
Growth hormone deficiency (% patients receiving substitution)	68
Sex hormone deficiency (% of patients)	59
Men: testosterone (% of patients receiving substitution)	69
Premenopausal women, n = 8: estrogens (% of patients receiving substitution)	50
Postmenopausal women, n = 10: estrogens	NA
Desmopressin (% of patients)	17

Abbreviations: IQR: interquartile range; NA: not applicable.

Supplemental Table 2. Pharmacokinetic parameters for the three weight groups for plasma free cortisol.

Lower dose	50–74 kg n = 14	75–84 kg n = 9	85–100 kg n = 23	*P*-value
C_{max1} (nmol/L)	35.99 [25.73; 48.22]	33.39 [28.15; 55.96]	32.58 [21.67; 43.40]	0.611
AUC_1 (h*nmol/L)	104.52 [75.70; 129.87]	83.98 [70.90; 138.23]	85.91 [61.00; 116.66]	0.417
C_{max2} (nmol/L)	25.95 [19.80; 34.43]	20.64 [15.86; 32.31]	24.51 [18.29; 35.67]	0.683
AUC_2 (h*nmol/L)	79.56 [60.63; 111.01]	64.16 [36.21; 78.17]	74.02 [49.51; 93.87]	0.244
C_{max3} (nmol/L)	15.13 [10.70; 20.96]	10.56 [8.08; 15.30]	10.16 [7.43; 13.50]	0.025*
AUC_3 (h*nmol/L)	43.66 [37.40; 80.99]	28.92 [18.90; 46.67]	28.27 [23.72; 40.58]	0.020*
AUC_{24h} (h*nmol/L)	236.30 [173.18; 361.36]	177.43 [126.18; 258.96]	192.01 [128.15; 251.35]	0.248
Higher dose	**50–74 kg n = 14**	**75–84 kg n = 9**	**85–100 kg n = 23**	***P*-value**
C_{max1} (nmol/L)	71.51 [50.71; 77.79]	81.43 [67.85; 113.29]	64.72 [52.75; 77.95]	0.175
AUC_1 (h*nmol/L)	200.96 [152.90; 222.44]	225.51 [171.78; 269.68]	187.37 [142.51; 247.64]	0.441
C_{max2} (nmol/L)	53.19 [38.29; 57.09]	48.12 [36.33; 60.22]	54.86 [44.10; 68.87]	0.450
AUC_2 (h*nmol/L)	143.14 [113.08; 164.33]	121.94 [91.88; 157.73]	156.03 [123.60; 196.91]	0.322
C_{max3} (nmol/L)	28.52 [19.97; 30.46]	24.66 [18.56; 29.96]	21.68 [16.44; 28.03]	0.243
AUC_3 (h*nmol/L)	83.87 [67.18; 97.12]	64.65 [47.91; 92.19]	67.33 [49.44; 91.36]	0.204
AUC_{24h} (h*nmol/L)	421.63 [332.48; 478.43]	411.77 [311.72; 515.64]	416.46 [313.88; 538.62]	0.999

Abbreviations: C_{max1}: maximum concentration after the first administration; AUC_1: area under the curve after the first administration; C_{max2}: maximum concentrations after the second administration; AUC_2: area under the curve after the second administration; C_{max3}: maximum concentrations after the third administration; AUC_3: area under the curve after the third administration; AUC_{24h}: 24 hour area under the curve.

* Statistical significant difference between the lowest weight group (50–74 kg) and the highest weight group (85–100 kg).

Supplemental Table 3. Pharmacokinetic parameters for the three weight groups for plasma total cortisol.

Lower dose	50–74 kg n = 14	75–84 kg n = 9	85–100 kg n = 23	*P*-value
C_{max1} (nmol/L)	541.67 [438.42; 760.91]	521.54 [464.69; 639.89]	482.99 [353.08; 595.06]	0.187
AUC_1 (h*nmol/L)	1984.34 [1381.15; 2728.00]	1502.67 [1297.27; 2070.43]	1504.10 [1092.05; 1872.48]	0.144
C_{max2} (nmol/L)	483.15 [391.14; 702.84]	393.03 [264.56; 437.88]	386.16 [282.45; 558.23]	0.136
AUC_2 (h*nmol/L)	1634.98 [1141.14; 2686.29]	1144.10 [715.02; 1590.25]	1183.09 [954.31; 1720.02]	0.107
C_{max3} (nmol/L)	286.32 [187.56; 487.77]	179.35 [135.59; 266.48]	202.27 [147.55; 252.85]	0.016*
AUC_3 (h*nmol/L)	1223.90 [651.07; 2426.45]	535.60 [394.78; 1321.44]	662.24 [489.34; 1101.74]	0.054
AUC_{24h} (h*nmol/L)	4862.89 [3075.09; 8201.59]	3211.18 [2441.57; 4933.21]	3759.60 [2850.41; 4745.90]	0.128
Higher dose	**50–74 kg n = 14**	**75–84 kg n = 9**	**85–100 kg n = 23**	***P*-value**
C_{max1} (nmol/L)	759.62 [731.81; 925.33]	906.95 [673.64; 1156.61]	729.46 [646.69; 783.03]	0.148
AUC_1 (h*nmol/L)	2685.46 [2315.51; 3466.15]	3013.60 [2116.62; 3530.51]	2409.28 [2149.96; 2839.97]	0.312
C_{max2} (nmol/L)	677.53 [606.59; 824.15]	628.76 [412.69; 757.10]	687.50 [600.88; 760.96]	0.656
AUC_2 (h*nmol/L)	227.37 [1932.39; 3200.90]	1945.96 [1279.56; 2554.02]	2332.25 [1873.00; 2671.04]	0.402
C_{max3} (nmol/L)	392.70 [336.35; 561.90]	319.24 [219.71; 410.78]	328.46 [255.88; 380.34]	0.060
AUC_3 (h*nmol/L)	1598.97 [1282.61; 2916.63]	1138.32 [771.14; 1699.39]	1489.74 [918.88; 1859.88]	0.064
AUC_{24h} (h*nmol/L)	6561.79 [5548.51; 9391.69]	5974.13 [4167.31; 7783.91]	5880.28 [5022.00; 7101.21]	0.534

Abbreviations: C_{max1}: maximum concentration after the first administration; AUC_1: area under the curve after the first administration; C_{max2}: maximum concentrations after the second administration; AUC_2: area under the curve after the second administration; C_{max3}: maximum concentrations after the third administration; AUC_3: area under the curve after the third administration; AUC_{24h}: 24 hour area under the curve.

* Statistical significant difference between the lowest weight group (50–74 kg) and the highest weight group (85–100 kg)