EUR Research Information Portal

Optimizing the Timing of Highest Hydrocortisone Dose in Children and Adolescents With 21-Hydroxylase Deficiency

Published in:
Journal of Clinical Endocrinology and Metabolism

Publication status and date:
Published: 01/04/2022

DOI (link to publisher):
10.1210/clinem/dgab826

Document Version
Publisher's PDF, also known as Version of record

Document License/Available under:
CC BY-NC-ND

Citation for the published version (APA):
Schröder, M. A. M., Van Herwaarden, A. E., Span, P. N., Van Den Akker, E. L. T., Bocca, G., Hannema, S. E., Van Der Kamp, H. J., De Kort, S. W. K., Mooij, C. F., Schott, D. A., Straetemans, S., Van Tellingen, V., Van Der Velden, J. A., Sweep, F. C. G. J., & Claahsen-Van Der Grinten, H. L. (2022). Optimizing the Timing of Highest Hydrocortisone Dose in Children and Adolescents With 21-Hydroxylase Deficiency. *Journal of Clinical Endocrinology and Metabolism*, *107*(4), E1661-E1672. https://doi.org/10.1210/clinem/dgab826

Link to publication on the EUR Research Information Portal

Terms and Conditions of Use
Except as permitted by the applicable copyright law, you may not reproduce or make this material available to any third party without the prior written permission from the copyright holder(s). Copyright law allows the following uses of this material without prior permission:

- you may download, save and print a copy of this material for your personal use only;
- you may share the EUR portal link to this material.

In case the material is published with an open access license (e.g. a Creative Commons (CC) license), other uses may be allowed. Please check the terms and conditions of the specific license.

Take-down policy
If you believe that this material infringes your copyright and/or any other intellectual property rights, you may request its removal by contacting us at the following email address: openaccess.library@eur.nl. Please provide us with all the relevant information, including the reasons why you believe any of your rights have been infringed. In case of a legitimate complaint, we will make the material inaccessible and/or remove it from the website.

Erasmus University Rotterdam
Making Minds Matter

The Journal of Clinical Endocrinology & Metabolism, 2022, Vol. 107, No. 4, e1661–e1672
https://doi.org/10.1210/clinem/dgab826
Clinical Research Article

Clinical Research Article

Optimizing the Timing of Highest Hydrocortisone Dose in Children and Adolescents With 21-Hydroxylase Deficiency

Mariska A. M. Schröder,[1,2] Antonius E. van Herwaarden,[2] Paul N. Span,[3] Erica L. T. van den Akker,[4] Gianni Bocca,[5] Sabine E. Hannema,[6,7] Hetty J. van der Kamp,[8] Sandra W. K. de Kort,[9] Christiaan F. Mooij,[10] Dina A. Schott,[11] Saartje Straetemans,[12] Vera van Tellingen,[13] Janiëlle A. van der Velden,[1] Fred C. G. J. Sweep,[2] and Hedi L. Claahsen-van der Grinten[1]

[1]Amalia Children's Hospital, Department of Pediatrics, Radboud University Medical Center, 6500 HB Nijmegen, the Netherlands; [2]Department of Laboratory Medicine, Radboud Institute for Molecular Life Sciences (RIMLS), Radboud University Medical Center, 6500 HB Nijmegen, the Netherlands; [3]Radiotherapy & OncoImmunology Laboratory, Department of Radiation Oncology, Radboud Institute for Molecular Life Sciences (RIMLS), Radboud University Medical Center, 6500 HB Nijmegen, the Netherlands; [4]Department of Pediatrics, Division of Endocrinology, Erasmus MC, University Medical Center Rotterdam, 3000 DR Rotterdam, the Netherlands; [5]Beatrix Children's Hospital, Department of Pediatrics, University Medical Center Groningen, 9700 RB Groningen, the Netherlands; [6]Department of Pediatrics, Leiden University Medical Centre, 2300 RC Leiden, the Netherlands; [7]Department of Pediatric Endocrinology, Emma Children's Hospital, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam Gastroenterology Endocrinology Metabolism, 1007 MB Amsterdam, the Netherlands; [8]Wilhelmina Children's Hospital, Utrecht University Medical Center, 3584 EA Utrecht, the Netherlands; [9]Department of Pediatrics, Haga Teaching Hospital/Juliana Children's Hospital, 2545 AA The Hague, the Netherlands; [10]Department of Pediatric Endocrinology, Emma Children's Hospital, Amsterdam University Medical Centers, University of Amsterdam, 1105 AZ Amsterdam, the Netherlands; [11]Department of Pediatrics Endocrinology, Zuyderland medical center, 6419 PC Heerlen, the Netherlands; [12]Department of Pediatric Endocrinology, Maastricht university medical center, 6229 HX Maastricht, the Netherlands; and [13]Department of Pediatrics, Catharina Hospital, 5623 EJ Eindhoven, the Netherlands

ORCiD numbers: 0000-0003-2139-9076 (M. A. M. Schröder); 0000-0001-5352-9328 (E. L. T. van den Akker); 0000-0002-2665-8738 (G. Bocca); 0000-0002-8996-0993 (S. E. Hannema); 0000-0003-0181-0403 (H. L. Claahsen-van der Grinten).

Abbreviations: 17OHP, 17-hydroxyprogesterone; 21OHD, 21-hydroxylase deficiency; A4, androstenedione; ACTH, adrenocorticotropic hormone; BP, blood pressure; CAH, congenital adrenal hyperplasia; HC, hydrocortisone; HE, high evening treatment regimen; HM, high morning treatment regimen; HPA, hypothalamic-pituitary-adrenal; LC-MS, liquid chromatography–mass spectrometry; OR, odds ratio; URL, upper reference limit.

Received: 23 July 2021; Editorial Decision: 7 November 2021; First Published Online: 12 November 2021; Corrected and Typeset: 7 December 2021.

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA
© The Author(s) 2021. Published by Oxford University Press on behalf of the Endocrine Society.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

Downloaded from https://academic.oup.com/jcem/article/107/4/e1661/6426165 by guest on 15 February 2023

Abstract

Context: Hydrocortisone treatment of young patients with 21-hydroxylase deficiency (21OHD) is given thrice daily, but there is debate about the optimal timing of the highest hydrocortisone dose, either mimicking the physiological diurnal rhythm (morning), or optimally suppressing androgen activity (evening).

Objective: We aimed to compare 2 standard hydrocortisone timing strategies, either highest dosage in the morning or evening, with respect to hormonal status throughout the day, nocturnal blood pressure (BP), and sleep and activity scores.

Methods: This 6-week crossover study included 39 patients (aged 4-19 years) with 21OHD. Patients were treated for 3 weeks with the highest hydrocortisone dose in the morning, followed by 3 weeks with the highest dose in the evening (n = 21), or vice versa (n = 18). Androstenedione (A4) and 17-hydroxyprogesterone (17OHP) levels were quantified in saliva collected at 5 AM; 7 AM; 3 PM; and 11 PM during the last 2 days of each treatment period. The main outcome measure was comparison of saliva 17OHP and A4 levels between the 2 treatment strategies.

Results: Administration of the highest dose in the evening resulted in significantly lower 17OHP levels at 5 AM, whereas the highest dose in the morning resulted in significantly lower 17OHP and A4 levels in the afternoon. The 2 treatment dose regimens were comparable with respect to averaged daily hormone levels, nocturnal BP, and activity and sleep scores.

Conclusion: No clear benefit for either treatment schedule was established. Given the variation in individual responses, we recommend individually optimizing dose distribution and monitoring disease control at multiple time points.

Key Words: hydrocortisone, dosing, CAH, congenital adrenal hyperplasia, 21-hydroxylase deficiency

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive diseases caused by mutations affecting adrenal steroid biosynthesis. In most cases (> 90%), CAH is caused by 21-hydroxylase deficiency (21OHD) (1), resulting in impaired cortisol and, in the most severe cases, decreased aldosterone production. Owing to the diminished cortisol production, negative feedback toward the hypothalamus and pituitary gland is decreased, resulting in increased production of pituitary adrenocorticotropic hormone (ACTH) and consequently overproduction of adrenal precursor steroids and adrenal androgens (2).

Treatment of patients with classic CAH consists of chronic glucocorticoid and, when necessary, mineralocorticoid administration, aiming to replace the relative glucocorticoid deficiency and to suppress the ACTH-mediated hyperandrogenemia (3). Usually, supraphysiological doses are required to inhibit ACTH and consequently androgen production (4). Yet, it is recommended not to completely suppress adrenal steroid production to prevent the adverse effects of glucocorticoid overtreatment (5). Overtreatment is, among other things, associated with cardiovascular complications, whereas undertreatment results in signs of chronic hyperandrogenism, and may result in the development or progression of testicular adrenal rest tumors in men (6). Therefore, the balance between overtreatment and undertreatment is a challenge for every health care provider taking care of CAH patients, and often patients may experience both periods of overtreatment and undertreatment during the day. Adequacy of treatment can be monitored by salivary levels of the precursor steroid 17-hydroxyprogesterone (17OHP) and the adrenal androgen androstenedione (A4) (7). For children with 21OHD, hydrocortisone (HC) treatment is recommended on a thrice-daily schedule (5). However, insufficient data exist regarding the best timing of the highest HC doses. In healthy individuals, cortisol levels follow a circadian rhythm with nadir cortisol levels at night, which start to rise between 2 AM and 4 AM, peak around 7 AM, and gradually decline during the day (8). One of the treatment strategies is to give the highest dose of glucocorticoids in the morning (9, 10), mimicking this physiological circadian cortisol rhythm (11). An alternative treatment strategy is to give the highest dose in the evening, which is suggested to inhibit the increase of androgens in the early morning, when androgen levels are highest, more effectively (12, 13). A high dose of HC in the evening, however, may negatively influence sleep (14-16) and nocturnal blood pressure (BP) (17). Normally, BP drops during the night, but children and adolescents with 21OHD may experience an absence of this nocturnal dip (18, 19) and can have nocturnal hypertension

Downloaded from https://academic.oup.com/jcem/article/107/4/e1661/6426165 by guest on 15 February 2023

(19). This may be attributed to the high evening HC dose treatment regimen (17). Several studies have focused on the number of daily doses (20, 21), or on the choice of synthetic glucocorticoid (22, 23), but studies on the best timing of highest glucocorticoid dose are limited, despite both treatment regimens being widely used (24). German et al (10) evaluated morning vs evening administration of a high HC dose with respect to disease control, sleep pattern, and daytime activity in children with CAH in a 4-week crossover study. No difference in basal hormone levels and sleep or activity measures between the 2 treatment regimens were detected. However, hormone levels were measured only at 8 AM, which does not adequately reflect the hormonal status throughout the day (25, 26).

Therefore, the present study aims to evaluate 2 standard treatment timing strategies for hydrocortisone dosage—either highest dosage in the morning or highest dosage in the evening—with respect to biochemical disease control in the early morning, morning, afternoon, and evening in children and adolescents with 21OHD. Secondary objectives are the evaluation of the treatment regimens with respect to overnight BP, sleep, and daytime activity. Optimization of glucocorticoid timing efficacy, while keeping the total dose equivalent, will help prevent the overtreatment and undertreatment in children with 21OHD.

Materials and Methods

Inclusion of Participants

Patients with CAH due to classic 21OHD aged between 4 and 20 years were invited to participate in the study. Inclusion criteria were a diagnosis of classic 21OHD confirmed by hormonal and mutation analysis and receiving treatment with HC according to standard guidelines (27). Children needed to be able to collect saliva. Patients with chronic medication use other than HC and fludrocortisone (including oral contraceptive use) or patients with other forms of CAH than 21OHD were excluded from the study.

Study Design

A prospective crossover study with a total duration of 6 weeks was performed. Patients were treated with the highest dose of HC in the evening (eg, 25%-25%-50% or 30%-30%-40% of daily dose) for 3 weeks followed by 3 weeks of treatment with the highest dose of HC in the morning (eg, 50%-25-25% or 40%-30%-30%), or the other way around, starting with their individualized regular total dose and dose distribution. In other words, patients started the study with their regular individualized dosing pattern and after 3 weeks switched their morning and evening dose. At the end of the last week of each 3-week treatment period, patients collected saliva for 2 consecutive days, 4 times a day at 5 AM; 7 AM; 3 PM; and 11 PM. At the latter 3 time points, saliva was collected just before the administration of hydrocortisone (at 7 AM, 3 PM, and 11 PM). Hormonal control before the start of the study was documented and disease control (poor/adequate/overtreatment) during the study periods was determined using in-house reference values for 17OHP and A4 (more details below; manuscript submitted.). In case of illness or stress during the study period, patients were instructed to take an extra dose of HC or increase the glucocorticoid dose and to contact the responsible physician. In those cases, treatment periods were extended, and saliva collection was deferred.

Ethics

The study was approved by the institutional review board (CMO Radboudumc No. 2018-004802-24). Written informed consent was obtained from patients older than 12 years and from caretakers of children younger than 16 years. The study conforms to the principles set out in the World Medical Association Declaration of Helsinki.

Laboratory Measurements

The primary outcome measures of this study were salivary 17OHP and A4 levels at 4 time points during the last 2 consecutive days of each 3-week treatment period. A4 and 17OHP levels in collected saliva samples were all quantified at Radboud University Medical Center, Nijmegen, the Netherlands, using liquid chromatography–mass spectrometry (LC-MS) after solid-phase extraction. An 8-point calibration series of A4 (0.038-82.5 nmol/L) and 17OHP (0.046-100 nmol/L) were used. Samples were homogenized by sonification and internal standards (IsoSciences) were added. Solid-phase extraction was performed using Oasis MCX 1-cc cartridges (Waters Corp). Columns were preequilibrated with methanol:isopropanol (95:5) and washed with 1 mL H_2O. After application of the samples, columns were washed with H_2O:NH_4OH (95:5) and methanol:H_2O:formic acid (20:78:2). Samples were eluated in methanol, dried under a stream of N_2 gas, and reconstituted in 30% methanol. Samples were injected into an Agilent Technologies 1290 Infinity UHPLC system, equipped with BEH C18 column (Waters Corp). Mobile phases were run in gradient with increasing methanol concentration. Retention time was 3.6 (A4) and 5.0 (17OHP) minutes. An Agilent Technologies 6490 Triple Quad LCMS operated in electrospray positive ion mode. Two mass transitions were monitored per analyte and internal standards. The first mass transition was used for quantification, and a second mass transition was used for confirmation. The

Downloaded from https://academic.oup.com/jcem/article/107/4/e1661/6426165 by guest on 15 February 2023

LC-MS/MS method is described in more detail elsewhere (manuscript submitted). All collected saliva samples per patient were quantified in the same run. The intra-assay variation is 2.5% and 2.5% for A4 and 17OHP, respectively.

Hormonal control during the morning, afternoon, and evening was classified based on the 17OHP and A4 levels (average of 2 days) and in-house reference values for prepubertal (Tanner of 1) and pubertal/adult patients (manuscript submitted), during each treatment period: 1) 17OHP above the upper reference limit (URL) and A4 below the URL, suggesting optimal control; 2) both 17OHP and A4 below the URL, suggesting overtreatment; and 3) both 17OHP and A4 above the URL, suggesting undertreatment.

Blood Pressure and Daily Sleep and Activity Scores

To study the influence of the 2 treatment regimens on nocturnal BP, a BP measurement was performed overnight for 1 night in the last week of each treatment period using an ambulant BP monitor (Spacelabs Healthcare), with approximately 1-hour interval measurements. To study the effects of treatment regimen on daytime activity and on sleep, participants or their caretakers gave a daily sleeping score between 0 and 5 (the higher the better) and daily morning, afternoon, and evening activity scores between 0 and 10 (the higher the better), each day during the entire study period. When participants reported more than one sleeping score for a night, sleeping scores were set to "not applicable" (NA).

Statistical Analysis

For the calculation of the minimum effect of interest, G*Power software was used (28). Morning A4 levels were used as the primary outcome measure. Assuming a within-patient SD of 0.15 nmol/L, we estimated that with a sample size of 39 participants a difference of 0.07 nmol/L would be detected (α = .05 [2-sided], power = 80%). Statistical analysis was performed in R (R Core Team; 2019). Because of nonnormality, hormone data were logarithmically transformed. The effect of treatment on averaged daily hormone levels was evaluated using linear mixed-effect models, with treatment regimen (high morning or high evening dose) and period (first or second) as fixed independent variables with a random patient effect to allow the patients' baseline values to vary. To study differences in single 17OHP and A4 levels at different time points, a time point variable (early morning, morning, afternoon, evening) and an interaction term between time point and treatment was added to the model. Evaluation of a potential interaction between treatment regimen and period confirmed the absence of a carryover effect and, therefore, the interaction was removed from the final model. Residual plots were inspected for deviations from normality or homoscedasticity. Significance of variables was evaluated using analysis of variance with Kenward-Roger approximation. In case of significant main effects or interactions, pairwise comparisons were performed using Tukey post hoc testing. Estimates with 95% CI are presented in log scale for hormonal data. To ease interpretation, raw nontransformed data are described as median with interquartile ranges. Unplanned exploratory subgroup analyses were performed to evaluate interactions between treatment regimen (high morning vs high evening) and Tanner, pubertal state (Tanner = 1 vs Tanner > 1), sex, or disease control (undertreated/optimal/overtreated) on daily average 17OHP and A4 levels. In addition, interactions between treatment regimen, time point (5 AM, 7 AM, 3 PM, 11 PM), and the aforementioned parameters on single 17OHP and A4 levels were evaluated. It should be noted that the study was not powered for these subgroup analyses. Differences in patients' mean nocturnal systolic and diastolic BP and differences in patients' mean activity scores (1-10) between treatment regimens were studied using linear mixed-effect models, with treatment regimen, period, and time points (for activity scores) as fixed effects and random patient effect, followed by Tukey post hoc testing. The influence of treatment regimen on sleep scores (1-5) was evaluated using cumulative link mixed-model analysis, also known as ordinal logistic regression, with treatment regimen and period as fixed effects and random patient effect. Statistical significance was considered at *P* values less than .05.

Results

Clinical Characteristics of Participants and Adverse Events

Forty patients were enrolled in the study, of whom 39 patients (median age 12 years; range, 4-19 years) completed the study, including 22 boys and 17 girls (Table 1). One patient prematurely quit the study because of general malaise not related to the study. Thirty-six patients had the salt-wasting form of CAH and received fludrocortisone treatment. Thirteen (33% [13 of 39]) participants had prepubertal Tanner stage during the study period. Twenty-one of the 39 patients (54%) started with the highest HC dose in the morning, and the remaining 18 patients started with the highest HC dose in the evening. The median dose distribution over the day was 48% in the morning, 25% in the afternoon, and 29% in the evening, when taking the highest dose in the morning. All but one patient received HC treatment thrice daily. This one patient received hydrocortisone at 7 AM, 1 PM, 6 PM, and 11 PM. For this patient,

Downloaded from https://academic.oup.com/jcem/article/107/4/e1661/6426165 by guest on 15 February 2023

Table 1. Clinical characteristics of 39 participants with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency

	Characteristics
Age, y	12 (4-19)
Sex, M/F	22/17
CAH type, SW/SV	36/3
Tanner stage, G/M	13× T1; 6× T2; 4× T3; 2× T4; 14× T5
Total daily HC dose (n = 39)	16 mg (7-30)
Total HC dose/m^2/d (n = 39)	11.7 mg (7.4-17.8)
Highest dose, morning/evening	21/18
Dose distribution, HM	48% (33-57); 25% (13-53); 29% (13-38)
Dose distribution, HE	29% (13-38); 25% (13-53); 48% (33-57)

Characteristics are summarized by median with ranges, categorical identifiers, or percentages.
Abbreviations: CAH, congenital adrenal hyperplasia; F, female; HC, hydrocortisone; HE, high evening treatment regimen; HM, high morning treatment regimen; M, male; SV, simple-virilizing; SW, salt-wasting.

afternoon and evening steroid hormone levels were not included in the analysis. If saliva was not collected at the right time points, steroid hormone levels were also set to not applicable. In total, 68 (10.9%) 17OHP steroid hormone levels were missing (19 during the high morning regimen [HM] and 49 during high evening regimen [HE]) and 66 (10.6%) A4 steroid hormone levels were missing (19 during HM and 47 during HE). Most steroid hormone levels were missing for the 5 AM saliva collection (n = 24). No severe adverse events occurred during the study period.

High Morning Dose Results in Lower Afternoon 17-Hydroxyprogesterone (17OHP) and Androstenedione Levels but Higher Early Morning 17OHP Levels

The primary aim was to define whether the different HC regimens resulted in different salivary 17OHP and A4 levels throughout the day, which were measured at 4 different time points (Table 2): the early morning (4.58 AM ± 0.31 (mean ± SD)), morning (7.40 AM ± 0.58), afternoon (2.40 PM ± 1.20), and in the evening (10.03 PM ± 1.33), before administration of HC treatment, which was given in the morning (7.50 AM ± 0.59 [average of last 2 days]), afternoon (2.48 PM ± 1.11), and evening (10.23 PM ± 1.30). When data from the 4 time points were averaged, patients' mean A4 levels and mean 17OHP levels were not different between the HM vs HE treatment regimen (estimate A4 = –0.04; CI, –0.25 to 0.16; n = 35 during HM and n = 31 during HE; estimate 17OHP = 0.001; CI, – 0.29 to 0.29; n = 35 during HM and n = 31 during HE). Yet, significant interactions were observed between treatment regimen and time point on both 17OHP and A4 levels (both $P < .001$), indicating that the effect of the treatment regimen differed between time points. While an HE dose resulted in lower levels of 17OHP (Tukey post hoc testing; $P < .001$) and, although statistically insignificant, lower levels (differences within patients) of A4 ($P = .12$) in the early morning compared to the HM dose regimen, an HM dose resulted in significantly lower levels of 17OHP ($P = .04$) and A4 ($P = .01$) in the afternoon compared to the HE dose regimen. No differences were observed in salivary 17OHP or A4 levels between treatment regimens in the morning or evening. Median concentrations for each treatment regimen and time point, as well as the β-coefficients (estimates) with CIs, are displayed in Table 2.

Variability between patients was high (Fig. 1A and 1B). No associations between Tanner stage, disease control 4 to 484 days before the study, disease control during the study period, dose/m^2, or sex and treatment effect (differences in mean 17OHP or A4 levels or single 17OHP or A4 levels throughout the day between treatment regimens) were observed (data not shown). For 17OHP no statistically significant difference was observed between periods (estimate [first vs second] = –0.14 (–0.31 to 0.04); $P = .13$). A4 levels were slightly lower during the first study period (estimate [first vs second] = –0.14 (–0.23 to –0.04); $P = .005$), regardless of dosing order, compared to the second study period.

Morning Hormonal Control Was Comparable Between Treatment Regimens

Based on the 17OHP and A4 levels and in-house reference values, hormonal control in the morning, afternoon, and evening was determined for each patient during both treatment periods. During the morning of the HM and HE treatment period, respectively, 19 and 18 patients were treated optimally, 4 and 3 patients were overtreated, and 16 and 15 patients were undertreated (Table 3). During the HE treatment period, 3 patients' hormonal control could not be determined. The stronger hormone suppression in the afternoon when receiving the higher HC dose in the morning vs the evening was reflected in hormonal control in the afternoon; 5 patients were optimally treated when receiving the highest dose in the morning but undertreated when receiving the highest dose in the evening (see Table 3). In addition, 2 patients were overtreated when receiving the highest dose in the morning but undertreated when receiving the highest dose in the evening, and 3 patients were overtreated when receiving the highest dose in the morning but optimally treated when receiving the highest dose in the evening. Contrarily, no patients were optimally treated during the HE treatment period but undertreated during

Downloaded from https://academic.oup.com/jcem/article/107/4/e1661/6426165 by guest on 15 February 2023

Table 2. Average of salivary 17-hydroxyprogesterone and A4 concentrations (nmol/L; median and interquartile range) measured in early morning (~ 5 AM), morning (~ 7 AM), afternoon (~ 3 PM), and evening (~ 11 PM) the last 2 consecutive days of each treatment regimen, that is, highest dose in morning (HM) or highest dose in evening (HE), together with the median difference between treatment regimens (HM vs HE)

	17OHP				
Time	HM	HE	Difference (HM – HE)	*P*	Estimates
5 AM	0.566 (0.204 to 1.252)	0.250 (0.045 to 0.745)	0.181 (0.001 to 0.526)	< .01	0.89 (0.35 to 1.43)
7 AM	1.357 (0.537 to 3.814)	1.909 (0.738 to 2.753)	–0.062 (–0.376 to 0.731)	≥ .999	0.03 (–0.49 to 0.55)
3 PM	0.518 (0.084 to 1.748)	0.786 (0.424 to 2.045)	–0.177 (–0.565 to 0.011)	.04	–0.54 (–1.06 to –0.02)
11 PM	0.078 (0.024 to –0.167)	0.100 (0.040 to 0.164)	–0.008 (–0.064 to 0.027)	≥ .999	–0.09 (–0.61 to 0.44)
	A4				
Time	HM	HE	Difference (HM – HE)	*P*	Estimates
5 AM	0.162 (0.062 to 0.418)	0.188 (0.058 to 0.341)	0.021 (–0.051 to 0.112)	.12	0.26 (–0.03 to 0.56)
7 AM	0.232 (0.100 to 0.689)	0.381 (0.165 to 0.701)	–0.026 (–0.081 to 0.064)	≥ .999	–0.02 (–0.30 to 0.26)
3 PM	0.121 (0.055 to 0.352)	0.281 (0.088 to 0.469)	–0.050 (–0.194 to 0.010)	.01	–0.33 (–0.62 to –0.04)
11 PM	0.065 (0.022 to 0.198)	0.096 (0.031 to 0.252)	–0.013 (–0.037 to 0.018)	.99	–0.08 (–0.37 to 0.21)

Differences between single logarithmically transformed 17OHP and A4 levels at the different time points were tested using linear mixed model analysis, followed by Tukey post hoc testing of the interaction term (treatment × time point). Estimates for HM vs HE regimen with 95% CI are in log scale.
Abbreviations: 17OHP, 17-hydroxyprogesterone; A4, androstenedione; HE, high evening treatment regimen; HM, high morning treatment regimen.

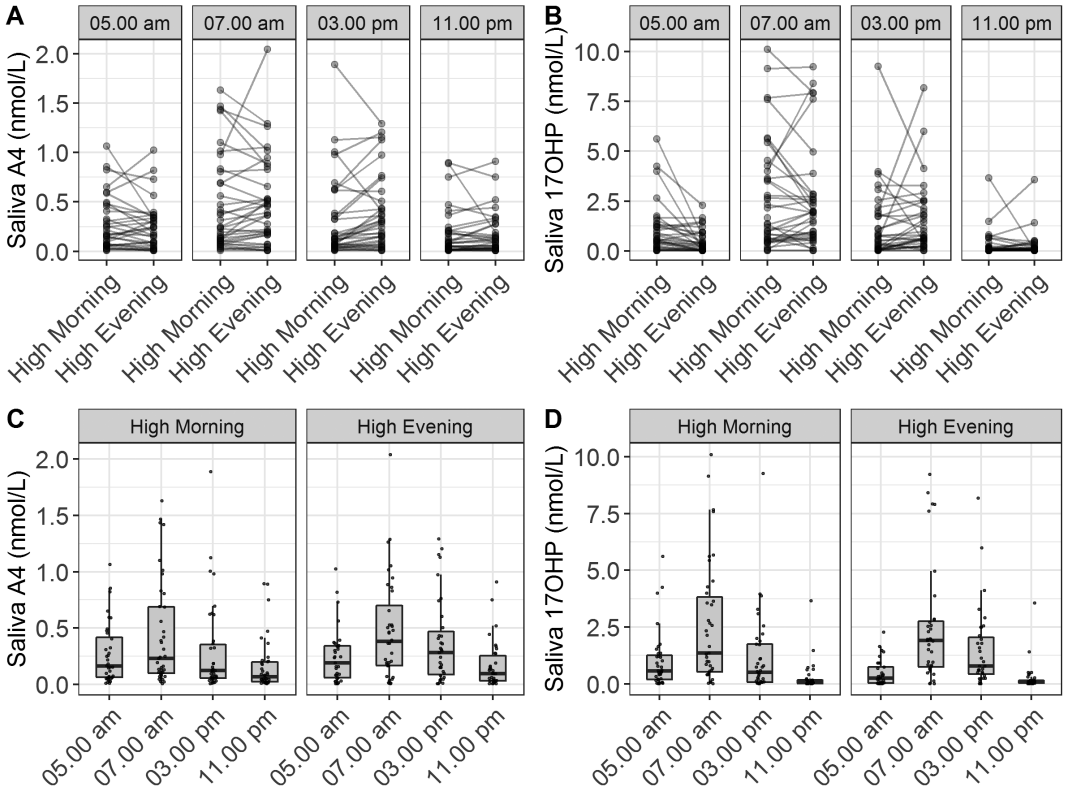

Figure 1. Two representations of the androstenedione (A4) (left panels) and 17-hydroxyprogesterone (17OHP) (right panels) data, A and B presenting the average differences (day 20 and day 21) between 2 treatment regimens at 4 time points for each patient. C and D present the pattern of A4 and 17OHP levels over the day for both treatment regimens.

Downloaded from https://academic.oup.com/jcem/article/107/4/e1661/6426165 by guest on 15 February 2023

Table 3. Hormonal control determined in morning, afternoon, and evening, during the high morning and high evening dose treatment periods

		Morning				
		HE				
		Undertreatment	Optimal	Overtreatment	ND	
HM	Undertreatment	13	2	0	1	16
	Optimal	2	15	1	1	19
	Overtreatment	0	1	2	1	4
		15	18	3	3	
		Afternoon				
		HE				
		Undertreatment	Optimal	Overtreatment	ND	
HM	Undertreatment	12	0	0	1	13
	Optimal	5	9	1	2	17
	Overtreatment	2	3	1	1	7
	ND	0	1	0	1	2
		19	13	2	5	
		Evening				
		HE				
		Undertreatment	Optimal	Overtreatment	ND	
HM	Undertreatment	7	1	1	1	10
	Optimal	2	10	1	4	17
	Overtreatment	0	4	5	1	10
	ND	0	1	0	1	2
		9	16	7	7	

Hormonal control was evaluated based on 17OHP and A4 levels at 7 AM, 3 PM, and 11.00 PM (average of consecutive days) with respect to in-house reference values, where 17OHP above the URL and A4 below the URL indicates optimal control, both 17OHP and A4 below the URL suggests overtreatment, and both 17OHP and A4 above the URL suggests undertreatment. In case of missing values, hormonal control could not be determined (ND).
Abbreviations: 17OHP, 17-hydroxyprogesterone; A4, androstenedione; HE, high evening treatment regimen; HM, high morning treatment regimen; URL, upper reference limit.

the HM treatment period and no patients were overtreated during the HE treatment period but undertreated during the HM treatment period. Yet, one patient was optimally treated in the afternoon during the HM regimen but overtreated during the HE regimen. Hormonal control could not be determined for the 5 AM time point.

Diurnal Variation in 17-Hydroxyprogesterone and Androstenedione Levels

Patients showed a circadian rhythm of 17OHP and A4 levels, with the highest levels in the morning, lower levels in the afternoon, and lowest levels in the evening (statistically significant differences between all time points; $P < .01$). Interestingly, this circadian rhythm was observed during both treatment periods (Fig. 1C and 1D), although it was less pronounced for the HE dose regimen. A4 levels were not statistically significantly higher in the morning vs the afternoon during the HE regimen (Tukey post hoc testing; $P = .053$).

Treatment Regimen Does Not Affect Sleep and Activity Scores

The secondary aim of this study was to evaluate whether the different treatment regimens affected sleep and daily activity rating. Patients reported sleep scores for each night during the entire study period. In total, 130 (7.9%) sleeping scores were missing, of which 55 were missing during the last week of the treatment periods. Overall, treatment regimen did not affect sleep rating (Fig. 2A; odds ratio [OR] = 0.93; $P = .50$). When concentrating only on the last week of each treatment period, patients seemed more likely (OR = 1.43) to give lower sleeping scores during the HE regimen, but this was not statistically significant ($P = .07$; Fig. 2B and 2C). Patients reported daily activity scores (from

Downloaded from https://academic.oup.com/jcem/article/107/4/e1661/6426165 by guest on 15 February 2023

1 to 10) for each morning, afternoon, and evening during the entire study period. In total, 88 (1.8%) activity scores were missing. Patients' mean activity scores were highest during the afternoon (estimated marginal mean = 6.6; 95% CI, 6.2-7.0), followed by the evening (6.2 [5.7-6.6]) and the morning (5.9 [5.0-6.4]) (Fig. 2D). Patients' mean activity scores differed significantly between the morning and the afternoon (estimate = –0.68 [–0.91 to –0.45]; $P < .01$) and between the afternoon and the evening (estimate = 0.46 [0.23-0.69]; $P < .01$), but did not significantly differ between the morning and evening (estimate = –0.22 [–0.46 to 0.01]; $P = .06$). No differences were observed in mean activity scores between the treatment regimens (estimate = –0.08 (–0.239 to 0.08]; see Fig. 2D).

Treatment Does Not Affect Nocturnal Blood Pressure

Ambulatory overnight BP monitoring during the last week of both treatment periods was completed by 36 patients but sufficient measurements (at least 5 data points) for both treatment periods were collected for 26 patients. In addition, 6 patients obtained enough data points only during the HM dose regimen (n = 32) and 4 patients collected sufficient data points only during the HE dose regimen (n = 30). Between the HM dose and HE dose regimen, no statistically significant differences were observed in mean overnight diastolic BP (estimate = 0.45 [–1.67 to 2.57]; $P = .67$) or mean systolic BP (estimate = –0.337 [–2.69 to 2.01]; $P = .77$).

Discussion

This study aimed to evaluate the timing of highest glucocorticoid dose with respect to hormonal control, overnight BP, sleep, and daytime activity in children and adolescents with 21OHD. We showed that whereas an HE dose resulted in lower levels of 17OHP in the early morning, an HM dose resulted in lower levels of 17OHP and A4 in the afternoon. Despite the early morning 17OHP suppression with the HE dose regimen, the suppressive effect was no longer observed in the morning (~ 7 AM), stressing the

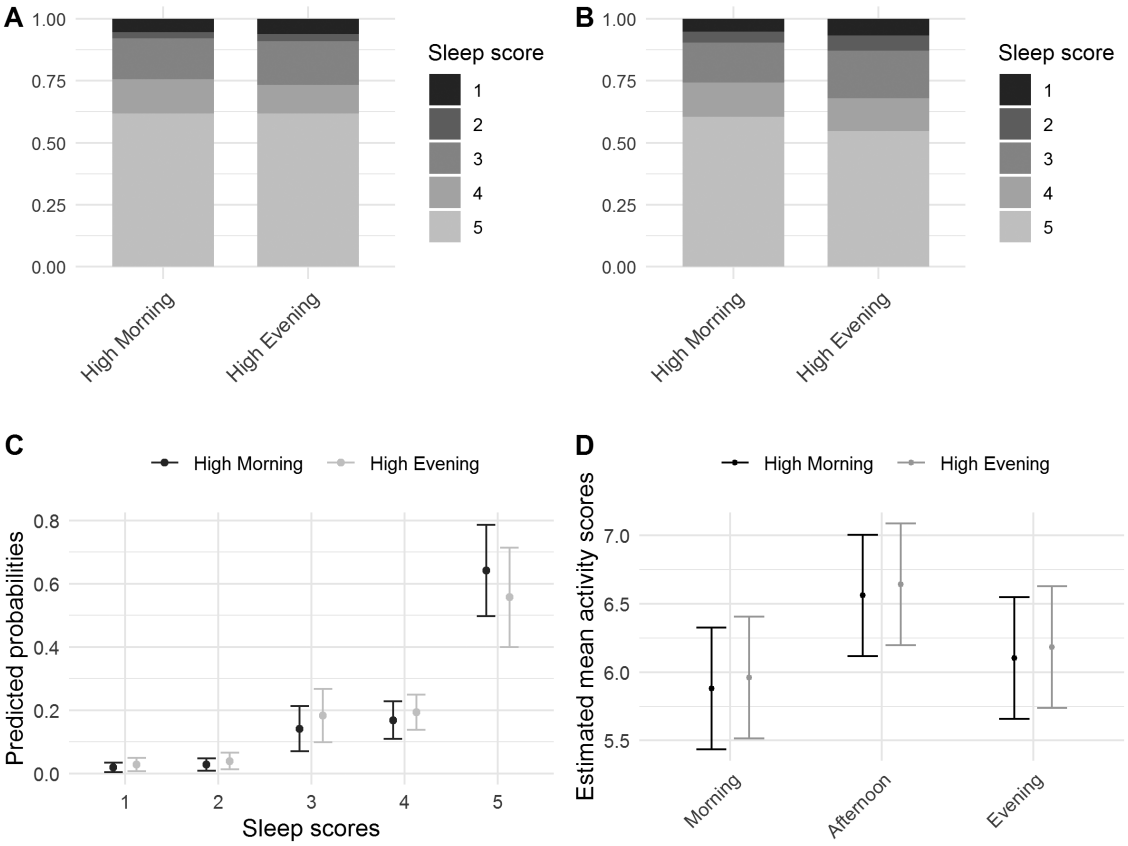

Figure 2. Sleep and activity scores in children and adolescents with 21-hydroxylase deficiency (21OHD) (n = 39) when either receiving the highest hydrocortisone dose in the morning (high morning) or evening (high evening). A and B present the cumulative proportions of sleep scores given during A, the complete 6-week study period, or B, during only the last week of each treatment period. C, Influence of treatment regimen on the probability (predicted probabilities with 95% CI) of sleep rating during the last week of each period was tested using cumulative link mixed model analysis. D, Differences in mean activity scores over the day (morning/afternoon/evening), presented as estimated marginal means with 95% CI, and influence of treatment regimen on mean activity scores were tested using linear mixed effect regression analysis followed by Tukey post hoc testing.

Downloaded from https://academic.oup.com/jcem/article/107/4/e1661/6426165 by guest on 15 February 2023

importance of giving the evening dose as late as possible and the morning dose as early as possible. Androgen suppression by HC lasts for 6 to 8 hours after evening-dose administration, after which the levels start to rise again (9, 29), explaining why we and German et al (10) did not observe a difference in morning hormone levels (7 AM or 8 AM) between the 2 treatment strategies. The HM dose regimen resulted in lower androgen levels in the afternoon. Overall, no difference in mean steroid levels throughout the day was observed between the treatment regimens. Importantly, the higher evening dose, which was given relatively late in this study (~ 10.23 PM), did not substantially affect sleep rating, daily activity scores, or nocturnal BP, and thus no treatment regimen was found to be superior.

Previously, it was postulated that a higher evening dose is not expected to inhibit the early morning rise in adrenal steroid levels, even when the evening dose is given late at night (30, 31). However, here we show that a higher evening dose better suppresses the 17OHP levels in the early morning, although the inhibition of A4 was less pronounced. This discrepancy between 17OHP and A4 might be explained by differences in the half-maximal inhibitory concentration values of hydrocortisone for 17OHP and A4, described by Al-Kofahi et al (26), which suggest that HC is a more potent inhibitor of 17OHP than A4 production. Nonetheless, a higher morning dose resulted in significantly lower levels both of 17OHP and A4 in the afternoon, suggesting a variability in glucocorticoid receptor sensitivity during the day, together with the physiological decrease of ACTH during the day. It could be speculated that suppression of the hypothalamic-pituitary-adrenal (HPA) axis in the early morning is more effective to achieve optimal hormonal control compared to the afternoon since the HPA axis is more active in the early morning. The interpatient variability in 17OHP and A4 levels—both in the early morning and in the afternoon—was high. While some patients displayed differences in 17OHP and A4 concentrations when treated with the different treatment regimens, other patients did not. This variability may be due to large interindividual differences in pharmacokinetic parameters (32-34), as subgroup analysis (interaction between treatment and eg, treatment dose per meters squared, tanner staging, or sex) could not explain this variability. For patients with higher clearance of HC, a relatively higher dose (either morning or evening) may, in contrast to patients with lower clearance, not result in lower levels of 17OHP and A4 6 or 8 hours after HC administration. On the other hand, this study was not powered for these subgroup analyses. The study cohort was quite heterogeneous, and it could be speculated that a more homogeneous study cohort may present differences between the treatment regimens and that an even bigger sample size may (hypothetically) allow the identification of patients that do benefit from one of the dosing strategies. Nonetheless, we did not observe trends suggesting particular patient groups benefiting from one or the other regimen.

In healthy children, 17OHP levels follow a circadian rhythm with highest levels in the morning, lower levels at noon, and lowest levels in the evening (35). We and others (36) showed that this circadian rhythm in 17OHP and A4 is still present in patients with 21OHD treated with dexamethasone and/or HC. Interestingly, this circadian rhythm was present during both treatment strategies, which might suggest that the intrinsic circadian regulation of 17OHP and A4 production is stronger than the exogeneous effects of HC. The circadian pattern was more prominent when the highest dose was administered in the morning vs the evening. For 17OHP, this circadian pattern was clearer than for A4, which complements previous research (36). The importance of a circadian rhythm in patients with 21OHD may be deduced from the improved quality of life in poorly controlled 21OHD patients receiving subcutaneous infusion of HC that mimic the physiological levels of cortisol (37).

In this study we made use of ambulatory BP measurement, which has been shown to be accurate and well tolerated in children, and may avoid "white-coat hypertension" (38). As we wanted to assess possible differences in cardiovascular risk between HC administration regimens, we were specifically interested in nocturnal BP because this is superior to daytime BP in predicting cardiovascular risk (38). Previously, we did not find associations between nocturnal BP and dosage of hydrocortisone or fludrocortisone (39). Elevated 24-hour diastolic and systolic BP levels and elevated overnight systolic BP were, however, reported in a small study of 6 children with 21OHD (aged 5.0-9.7 years) when patients received the highest dose in the evening (7 PM) (17), suggesting an effect on cardiovascular risk depending on HC treatment regimens. This could not be confirmed by our study presented here. Our study showed no difference in mean nocturnal BP between the treatment regimens in children with 21OHD. Although the sample size in the present study is larger, differences in relative evening dose during the HE vs HM regimens were greater in the study by Liivak and Tillmann (53% vs 17% of total dose) compared to our study (48% vs 29% of total dose) (17). Therefore, we conclude that relatively small elevations in evening HC dose do not translate into detectable differences in nocturnal BP.

Treatment regimen did not statistically significantly (P = .07) affect subjective sleep or activity rating, although sleep rating seemed lower during the last week of the HE dose regimen (OR = 1.43 for giving lower sleep score). Potentially, effects of an HE dose on sleep rating might be detected in a bigger cohort of patients. In addition, it may

Downloaded from https://academic.oup.com/jcem/article/107/4/e1661/6426165 by guest on 15 February 2023

be argued that glucocorticoid treatment may affect sleep quality unconsciously (16), without affecting subjective sleep rating. Nonetheless, our results are in line with the study by German et al (10), who did not find differences in sleep quality and activity between either treatment regimen in children with 21OHD. It remains to be studied whether the different dosing regimens affect long-term health outcomes.

Of interest is whether administration of the highest HC dose in the morning or in the evening may be more likely to result in overexposure to HC. As overnight cortisol levels are normally low, giving a high HC dose at bedtime potentially exposes the patient to unnecessarily high levels during this time frame (29). On the other hand, it could be hypothesized that, because of the diurnal rhythmicity of glucocorticoid receptor sensitivity (40-42), harmful effects of supraphysiological cortisol doses at night may potentially be limited. However, whether an equivalent glucocorticoid dose in the evening indeed results in a lower metabolic response than in the morning has, to our knowledge, not yet been studied in children with 21OHD.

Our study addresses the same research question as the study by German et al (10), but there are important differences between the present study and the study by German and colleagues. As stated by the authors, the primary end points of German et al were sleep and activity, which resulted in a relatively small sample size for hormonal analysis (n = 15). Importantly, we have quantified hormone levels at multiple time points per day, which better reflected hormonal status throughout the day. By including the 5 AM and 3 PM measurements, we were able to show that the HE regimen actually resulted in stronger suppression of 17OHP (ie, HPA axis suppression) in the early morning, and that an HM regimen resulted in stronger suppression in the afternoon.

Despite progress in the development of modified-release glucocorticoid substitutions, these are not yet available for pediatric patients with 21OHD. Therefore, until these formulations become available, we recommend that the dose distribution be individually optimized for each patient. Although overall, as a group, no treatment regimen was found to be superior, individual patients may benefit from one or the other dosing strategy. To optimize treatment, hormonal control should be monitored at multiple time points a day, preferably before taking the medication, because single measurements indicate hormonal control only at a specific time point and provide limited information on the patient's overall disease control (25, 26). The addition of an early morning salivary measurement is considered beneficial because it informs whether a higher evening dose results in better suppression of the early-morning surge in that patient. Nonetheless, it should be noted that, as the rise in ACTH starts between 2 and 4 in the morning, benefit from the HE dose in the early morning may not always be captured by the 5 AM measurement. In other words, while no difference in 17OHP and A4 levels may be evident from the 5 AM measurement, the HE dose may result in a period of higher androgen suppression before 5 AM. If, for a patient, the HM and HE treatment regimens result in indistinguishable total androgen exposure, it can be argued that a physiological pattern of HC intake is preferable.

In this study we measured steroids in saliva, because saliva collection is less stressful for children compared to venous blood sampling and, therefore, the steroid levels are less likely to be affected by specimen sampling (43). Levels of 17OHP and A4 have been reported to highly correlate between saliva and plasma samples (44, 45). Moreover, because androgens were measured in saliva, and BP was measured using an ambulatory BP monitor, no hospitalization was required for this study design, which minimized the burden for participants. This does, however, mean that compliance to treatment is not controlled. Yet, these patients were highly motivated and explicitly instructed to adhere to treatment administration at specific time points.

We do acknowledge several limitations in this study. Although the incorporation of multiple time points of androgen measurement over the day is a strength of this study, continuous monitoring (eg, 20-minute or 1-hour interval) throughout the day or the collection of 24-hour urine might have been even more informative because it would have allowed us to compare total androgen exposure between treatment regimens. Also, whether for example a higher morning dose adjusted the morning 17OHP and A4 levels faster or better was not determined here because steroid levels were quantified only before HC administration. However, the patients' load would have been much higher and would most likely have resulted in a much lower number of participants.

In conclusion, the HM dose and HE dose regimens were comparable with respect to averaged daily 17OHP and A4 levels, activity scores, sleeping scores, and nocturnal BP although they resulted in different exposure patterns to 17OHP and A4 throughout the day. We recommend individually determining the best timing of the highest HC dose based on levels of 17OHP and A4 at multiple time points during the day.

Acknowledgments

We would like to thank all patients and their caretakers for participation in this study. We acknowledge Spacelabs Healthcare and Itémedical for the use of their ambulant BP monitoring systems. We would like to thank Wendy Jansen and Nienke Sonneveld for their great support.

Financial Support: This work was supported by Innovatiefonds Zorgverzekeraars.

Downloaded from https://academic.oup.com/jcem/article/107/4/e1661/6426165 by guest on 15 February 2023

Clinical Trial Registration: EudraCT number 2018-004802-24 (registered 2020-08-12).

Additional Information

Correspondence: Hedi L. Claahsen-van der Grinten, MD, PhD, Amalia Children's Hospital, Radboud University Medical Center, Department of Pediatrics, Geert Grooteplein Zuid 10, 6500 HB, Nijmegen, the Netherlands. Email: Hedi.Claahsen@radboudumc.nl.

Disclosures: The authors have nothing to disclose.

Data Availability: Some or all data sets generated during and/or analyzed during the present study are not publicly available but are available from the corresponding author on reasonable request.

References

1. Speiser PW, White PC. Congenital adrenal hyperplasia. *N Engl J Med*. 2003;**349**(8):776-788.
2. White PC, Speiser PW. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Endocr Rev*. 2000;**21**(3):245-291.
3. Claahsen-van der Grinten HL, Stikkelbroeck NM, Otten BJ, Hermus AR. Congenital adrenal hyperplasia—pharmacologic interventions from the prenatal phase to adulthood. *Pharmacol Ther*. 2011;**132**(1):1-14.
4. Speiser PW, Azziz R, Baskin LS, et al; Endocrine Society. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2010;**95**(9):4133-4160.
5. Speiser PW, Arlt W, Auchus RJ, et al. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2018;**103**(11):4043-4088.
6. Han TS, Walker BR, Arlt W, Ross RJ. Treatment and health outcomes in adults with congenital adrenal hyperplasia. *Nat Rev Endocrinol*. 2014;**10**(2):115-124.
7. de Groot MJ, Pijnenburg-Kleizen KJ, Thomas CM, et al. Salivary morning androstenedione and 17α-OH progesterone levels in childhood and puberty in patients with classic congenital adrenal hyperplasia. *Clin Chem Lab Med*. 2015;**53**(3):461-468.
8. Porter J, Blair J, Ross RJ. Is physiological glucocorticoid replacement important in children? *Arch Dis Child*. 2017;**102**(2):199-205.
9. Charmandari E, Johnston A, Brook CG, Hindmarsh PC. Bioavailability of oral hydrocortisone in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Endocrinol*. 2001;**169**(1):65-70.
10. German A, Suraiya S, Tenenbaum-Rakover Y, Koren I, Pillar G, Hochberg Z. Control of childhood congenital adrenal hyperplasia and sleep activity and quality with morning or evening glucocorticoid therapy. *J Clin Endocrinol Metab*. 2008;**93**(12):4707-4710.
11. Linder BL, Esteban NV, Yergey AL, Winterer JC, Loriaux DL, Cassorla F. Cortisol production rate in childhood and adolescence. *J Pediatr*. 1990;**117**(6):892-896.
12. Rosenfield RL. Serum cortisol and 17-hydroxyprogesterone concentrations in children with classic congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2002;**87**(6):2993.
13. Merke DP. Approach to the adult with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2008;**93**(3):653-660.
14. Born J, Zwick A, Roth G, Fehm-Wolfsdorf G, Fehm HL. Differential effects of hydrocortisone, fluocortolone, and aldosterone on nocturnal sleep in humans. *Acta Endocrinol (Copenh)*. 1987;**116**(1):129-137.
15. Young AH, Sharpley AL, Campling GM, Hockney RA, Cowen PJ. Effects of hydrocortisone on brain 5-HT function and sleep. *J Affect Disord*. 1994;**32**(2):139-146.
16. Gillin JC, Jacobs LS, Fram DH, Snyder F. Acute effect of a glucocorticoid on normal human sleep. *Nature*. 1972;**237**(5355):398-399.
17. Liivak K, Tillmann V. 24-hour blood pressure profiles in children with congenital adrenal hyperplasia on two different hydrocortisone treatment regimens. *J Pediatr Endocrinol Metab*. 2009;**22**(6):511-517.
18. Roche EF, Charmandari E, Dattani MT, Hindmarsh PC. Blood pressure in children and adolescents with congenital adrenal hyperplasia (21-hydroxylase deficiency): a preliminary report. *Clin Endocrinol (Oxf)*. 2003;**58**(5):589-596.
19. de Silva KS, Kanumakala S, Brown JJ, Jones CL, Warne GL. 24-hour ambulatory blood pressure profile in patients with congenital adrenal hyperplasia—a preliminary report. *J Pediatr Endocrinol Metab*. 2004;**17**(8):1089-1095.
20. Ekman B, Bachrach-Lindström M, Lindström T, Wahlberg J, Blomgren J, Arnqvist HJ. A randomized, double-blind, crossover study comparing two- and four-dose hydrocortisone regimen with regard to quality of life, cortisol and ACTH profiles in patients with primary adrenal insufficiency. *Clin Endocrinol (Oxf)*. 2012;**77**(1):18-25.
21. Groves RW, Toms GC, Houghton BJ, Monson JP. Corticosteroid replacement therapy: twice or thrice daily? *J R Soc Med*. 1988;**81**(9):514-516.
22. Whittle E, Falhammar H. Glucocorticoid regimens in the treatment of congenital adrenal hyperplasia: a systematic review and meta-analysis. *J Endocr Soc*. 2019;**3**(6):1227-1245.
23. Han TS, Stimson RH, Rees DA, et al; United Kingdom Congenital adrenal Hyperplasia Adult Study Executive (CaHASE). Glucocorticoid treatment regimen and health outcomes in adults with congenital adrenal hyperplasia. *Clin Endocrinol (Oxf)*. 2013;**78**(2):197-203.
24. Ross RJ, Rostami-Hodjegan A. Timing and type of glucocorticoid replacement in adult congenital adrenal hyperplasia. *Horm Res*. 2005;**64**(Suppl 2):67-70.
25. Birkebaek NH, Hougaard DM, Cohen AS. Monitoring steroid replacement therapy in children with congenital adrenal hyperplasia. *J Pediatr Endocrinol Metab*. 2017;**30**(1):85-88.
26. Al-Kofahi M, Ahmed MA, Jaber MM, et al. An integrated PK-PD model for cortisol and the 17-hydroxyprogesterone and androstenedione biomarkers in children with congenital adrenal hyperplasia. *Br J Clin Pharmacol*. 2021;**87**(3):1098-1110.
27. Speiser PW, Arlt W, Auchus RJ, et al. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2018;**103**(11):4043-4088.
28. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 2007;**39**(2):175-191.

Downloaded from https://academic.oup.com/jcem/article/107/4/e1661/6426165 by guest on 15 February 2023

29. Fuqua JS, Rotenstein D, Lee PA. Duration of suppression of adrenal steroids after glucocorticoid administration. *Int J Pediatr Endocrinol*. 2010;**2010**:712549.
30. Charmandari E, Matthews DR, Johnston A, Brook CG, Hindmarsh PC. Serum cortisol and 17-hydroxyprogesterone interrelation in classic 21-hydroxylase deficiency: is current replacement therapy satisfactory? *J Clin Endocrinol Metab*. 2001;**86**(10):4679-4685.
31. HindMarsh PC, Geertsma K. *Congenital Adrenal Hyperplasia: A Comprehensive Guide*. Mica Haley; 2017.
32. Werumeus Buning J, Touw DJ, Brummelman P, et al. Pharmacokinetics of oral hydrocortisone—results and implications from a randomized controlled trial. *Metabolism*. 2017;**71**:7-16.
33. Hindmarsh PC, Charmandari E. Variation in absorption and half-life of hydrocortisone influence plasma cortisol concentrations. *Clin Endocrinol (Oxf)*. 2015;**82**(4):557-561.
34. Melin J, Parra-Guillen ZP, Michelet R, et al. Pharmacokinetic/pharmacodynamic evaluation of hydrocortisone therapy in pediatric patients with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2020;**105**(3):dgaa071.
35. Gröschl M, Rauh M, Dörr HG. Circadian rhythm of salivary cortisol, 17alpha-hydroxyprogesterone, and progesterone in healthy children. *Clin Chem*. 2003;**49**(10):1688-1691.
36. Young MC, Walker RF, Riad-Fahmy D, Hughes IA. Androstenedione rhythms in saliva in congenital adrenal hyperplasia. *Arch Dis Child*. 1988;**63**(6):624-628.
37. Nella AA, Mallappa A, Perritt AF, et al. A phase 2 study of continuous subcutaneous hydrocortisone infusion in adults with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2016;**101**(12):4690-4698.
38. Parati G, Stergiou G, O'Brien E, et al; European Society of Hypertension Working Group on Blood Pressure Monitoring and Cardiovascular Variability. European Society of Hypertension practice guidelines for ambulatory blood pressure monitoring. *J Hypertens*. 2014;**32**(7):1359-1366.
39. Mooij CF, van Herwaarden AE, Sweep FCGJ, et al. Cardiovascular and metabolic risk in pediatric patients with congenital adrenal hyperplasia due to 21 hydroxylase deficiency. *J Pediatr Endocrinol Metab*. 2017;**30**(9):957-966.
40. Xu RB, Liu ZM, Zhao Y. A study on the circadian rhythm of glucocorticoid receptor. *Neuroendocrinology*. 1991;**53**(Suppl 1):31-36.
41. Cardinal J, Pretorius CJ, Ungerer JP. Biological and diurnal variation in glucocorticoid sensitivity detected with a sensitive in vitro dexamethasone suppression of cytokine production assay. *J Clin Endocrinol Metab*. 2010;**95**(8):3657-3663.
42. Gratsias Y, Moutsatsou P, Chrysanthopoulou G, Tsagarakis S, Thalassinos N, Sekeris CE. Diurnal changes in glucocorticoid sensitivity in human peripheral blood samples. *Steroids*. 2000;**65**(12):851-856.
43. Vining RF, McGinley RA, Maksvytis JJ, Ho KY. Salivary cortisol: a better measure of adrenal cortical function than serum cortisol. *Ann Clin Biochem*. 1983;**20**(Pt 6):329-335.
44. Bacila I, Adaway J, Hawley J, et al. Measurement of salivary adrenal-specific androgens as biomarkers of therapy control in 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2019;**104**(12):6417-6429.
45. Otten BJ, Wellen JJ, Rijken JC, Stoelinga GB, Benraad TJ. Salivary and plasma androstenedione and 17-hydroxyprogesterone levels in congenital adrenal-hyperplasia. *J Clin Endocrinol Metab*. 1983;**57**(6):1150-1154.

Downloaded from https://academic.oup.com/jcem/article/107/4/e1661/6426165 by guest on 15 February 2023