Reduced slow-wave sleep and altered diurnal cortisol rhythms in patients with Addison's disease

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Abstract

Objectives: Cortisol plays a key role in initiating and maintaining different sleep stages. Patients with Addison's disease (AD) frequently report disrupted sleep, and their hydrocortisone medication regimes do not restore the natural diurnal rhythm of cortisol. However, few studies have investigated relations between sleep quality, especially as measured by polysomnographic equipment, and night-time cortisol concentrations in patients with AD.

Methods: We used sleep-adapted EEG to monitor a full night of sleep in seven patients with AD and seven healthy controls. We sampled salivary cortisol before bedtime, at midnight, upon awakening and at 30 min post waking.

Results: Controls had lower cortisol concentrations than patients before bedtime and at midnight. During the second half of the night, patient cortisol concentrations declined steeply, while control concentrations increased steadily. Whereas most controls experienced a positive cortisol awakening response, all patients experienced a decrease in cortisol concentrations from waking to 30 min post waking ($P=0.003$). Patients experienced significantly lower proportions of slow-wave sleep (SWS; $P=0.001$), which was associated with elevated night-time cortisol concentrations.

Conclusion: Overall, these results suggest that patients with AD demonstrate different patterns of night-time cortisol concentrations to healthy controls and that relatively elevated concentrations are associated with a reduction of SWS. These hormonal and sleep architectural aberrations may disrupt the routine sleep-dependent processes of memory consolidation, and hence, may explain, at least partially, the memory impairments often experienced by patients with AD.

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Introduction

Patients with Addison's disease (AD) have low plasma cortisol and aldosterone levels alongside high adrenocorticotrophic hormone (ACTH) levels and renin concentrations. A standard pharmacological intervention for these patients thus involves administration of a cortisol replacement (most often oral hydrocortisone or prednisone), along with an additional mineralocorticoid (e.g., fludrocortisone) to control sodium and potassium balance (1).

There are reciprocal relationships between hormone secretion and regulation of the sleep–wake cycle (2, 3).

Hormones of the hypothalamic–pituitary–adrenal (HPA) axis play a particularly important role in facilitating entry into, and in the timing and duration of, various sleep stages. Healthy individuals experiencing normal diurnal rhythmicity show relatively low cortisol, ACTH and corticotrophin-releasing hormone (CRH) concentrations during the early part of the night, along with high melatonin and growth hormone-releasing hormone (GHRH) concentrations. The high proportion of SWS during the early part of the night is stimulated by GHRH, via its effects on growth hormone (GH), and optimal

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cortisol levels experienced during early sleep probably enhance SWS through feedback inhibition of CRH (4, 5, 6, 7). In contrast, during the second half of the night, CRH stimulates cortisol and inhibits GH, thus creating a physiological environment that is more conducive for entry into, and longer duration of, rapid eye movement (REM) sleep (4, 8).

Standard GC replacement therapy does not restore the normal cortisol diurnal rhythm in patients with AD, resulting in alternating periods of sub- and supra-physiological concentrations. Hydrocortisone administration in the late afternoon or early evening leads to relatively elevated cortisol concentrations during the early part of the night (9), but the medication's short half-life results in cortisol deficiencies during the latter part of the night and the early morning (10). Given the importance of the HPA axis in sleep regulation (4), high night-time cortisol during the early hours of sleep, accompanied by high night-time ACTH and CRH, may be one reason why patients with AD frequently report experiencing disrupted, unrefreshing sleep (11, 12, 13).

However, only two published studies have examined the relationship between HPA axis activity and objectively measured sleep in patients with AD (14, 15). Although findings from both studies support the notion that HPA axis hypo- and hyperactivity in patients with AD alters sleep architecture, some methodological considerations temper any firm inferences. For instance, García-Borreguero *et al.* (14) did not report on the sleep architecture of their patients under basal conditions (i.e., when treated with usual replacement medication regimen), and Gillin *et al.* (15) was a small study in which patients ($n=3$) and controls ($n=5$) were not matched demographically (e.g. whereas patients' age range was 27–58, controls were aged <25 years).

Nonetheless, and especially in light of sizable literature describing relationships between irregular HPA axis activity and altered sleep architecture in both psychiatric and healthy samples (16, 17, 18), it is plausible to suggest that hypo- or hyperactivity of that endocrinological system might play a role in explaining why patients with AD self-report, and objectively experience, poorer sleep quality and more frequent sleep disturbances than healthy controls (11, 12, 14, 19).

The current study

Given the known associations between cortisol secretion and sleep architecture, and the limited research on sleep in patients with AD, we aimed to measure, using

polysomnographic techniques, the sleep quality and architecture of patients with AD who were on immediate-release hydrocortisone replacement therapy. Because we sought to maintain high levels of ecological validity, our design did not feature any manipulation of the patients' medication regimen; we describe possible mechanistic relations between cortisol concentrations and sleep architecture as they exist naturalistically, without any laboratory-based alteration, in this population.

We hypothesized that, compared to matched healthy controls, patients with AD, possibly due to their alternating periods of sub- and supra-physiological cortisol concentrations, will experience poorer sleep quality and disrupted sleep architecture. We also hypothesized that patients will, relative to controls, experience higher cortisol concentrations during the first half of the night, but lower concentrations during the second. Finally, we explored associations between cortisol concentrations and sleep patterns.

Subjects and methods

Participants

Our sample was seven adult patients with AD (recruited from the South African Addison's disease database (SAAD)) (20) and seven healthy community-dwelling adults (recruited from a university community and surrounding neighborhoods). All were part of a cohort participating in a research program investigating sleep, cognition and quality of life in AD (11, 19, 21). We matched the groups on age and sex distribution (each group included two men and five women), and on level of education (all had completed high school).

To avoid sleep-confounding effects of age, psychiatric status and hormonal variations (22, 23, 24), we included only (a) individuals between the ages of 18 and 55 years, (b) individuals who did not evince severe depressive symptomatology, (c) women who were pre-menopausal, not pregnant and not taking oral contraceptives. Healthy controls were required to be free of any chronic medical or psychiatric illnesses.

Measures

Screening instruments

A sociodemographic questionnaire obtained information about biographic variables and general medical history. For patients with AD, we extracted from the SAAD

database information regarding type and dosage of current medication and duration since diagnosis. The Mini International Neuropsychiatric Interview (MINI; English version 5.0.0) assessed current psychiatric status (25). The Beck Depression Inventory-Second Edition (BDI-II) measured current (2-week) intensity, severity and depth of depression (26). Individuals with BDI-II scores greater than 29 (indicating severe depression) were excluded from participation.

Subjective sleep assessment

The Pittsburgh Sleep Quality Index (PSQI) (27) measured self-reported sleep quality and disturbances over the month prior to completion. It comprises 19 items, each related to at least one of seven components (sleep quality, latency, duration, disturbances and efficiency; use of sleeping medication and daytime dysfunction due to disrupted sleep). The score on each component ranges from 0 to 3; hence, the total PSQI score ranges from 0 to 21, with higher scores representing poorer sleep quality marked by more disruptions. Individuals with a total score above 5 are considered to have poor sleep quality (28).

Objective sleep assessment

We used a Nihon Kohden sleep-adapted electroencephalograph (EEG). PSGs are a reliable means of measuring sleep efficiency and total sleep time and of classifying sleep stages (29). In the current study, the PSG measured brain activity, eye movements and muscle activity as the participant entered and passed through different sleep stages. We recorded data following the International 10–20 system (30).

Cortisol

We took salivary cortisol measurements as surrogate indicators of plasma cortisol concentration, collecting samples using Sarstedt salivettes (Sarstedt, Nümbrecht, Germany). The samples were analyzed using a competitive electrochemiluminescent immunoassay on the Roche Cobas 6000 (Roche Diagnostics GmbH) with a coefficient of variation of 4%.

Ethical approval and procedure

Research Ethics Committees from the University of Cape Town's Department of Psychology and Faculty of Health Sciences, both of which adhere to the Declaration

of Helsinki (31), approved the study procedures. All participants gave informed consent for collection, use and reporting of their data after being explained the full purpose of the study. No participant reported experiencing adverse events during the course of the study procedures. All data are held confidential, and participants' rights to anonymity and privacy were guaranteed and have been sustained.

We instructed those in the AD group to continue taking their hydrocortisone medication at their usual time and obtained verbal confirmation that they did so. All testing took place at a dedicated sleep laboratory. Sleep architecture was recorded in a sound-attenuated, light- and temperature-controlled room for two consecutive nights. The first of these was an adaptation night, which allowed participants to acclimatize to a new sleeping environment (29). Following precedent (32, 33, 34), data from this night were not analyzed.

Each participant arrived at the hospital at 20:30 h on the adaptation night. After reading and signing informed consent documents, they completed the PSQI and ESS, and were then attached to the PSG. At 22:00 h, lights were turned off and they were instructed to lie down and fall asleep as soon as possible. Lights were turned on at 06:00 h. Before leaving the laboratory, participants were instructed not to consume any caffeinated drinks during the day and to have their last meal 2–3 h before bedtime.

Participants returned to the sleep laboratory later that day, at around 20:00 h. The subsequent protocol was identical to that of the adaptation night, except that we sampled salivary cortisol four times (Fig. 1). Upon completion of these procedures, participants were debriefed and compensated.

Figure 1
Study procedure during the experimental night.

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Data management and statistical analyses

Subjective sleep

We used PSQI total score to represent overall self-reported sleep quality and calculated the proportion of participants within each group who could be classified as poor sleepers (i.e., with total score >5). We also used individual PSQI items to estimate four other subjective sleep outcome variables: total sleep time (in minutes), number of minutes spent awake after sleep onset, sleep efficiency (proportion of time in bed spent asleep) and sleep latency (length of time, in minutes, between going to bed and falling asleep).

Objective sleep

On the experimental night, each participant was evaluated continuously for approximately 8 h. We examined, for the full night and by half segments of the night, these 13 sleep-related outcome variables: sleep efficiency (proportion of time in bed spent asleep); sleep latency (length of time, in minutes, between lights out and falling asleep); REM latency (length of time, in minutes between lights out and onset of the first REM cycle); wake after sleep onset (WASO; the number of minutes spent awake in the period between sleep onset and final waking); time and proportion spent in each stage of sleep (N1, N2, SWS and REM) and number of awakenings (number of times the participant awoke for more than 1 min in the period between sleep onset and final waking).

We analyzed sleep data using Polysmith analysis software (Rosbach, Germany), and scored them according to standardized criteria outlined in the American Academy of Sleep Medicine Manual for the Scoring of Sleep and Associated Events (30). Sleep records were scored by M.H. and an independent qualified sleep technician, both of whom were blind to group assignment. For sleep-stage analysis, the two scorers had an epoch-by-epoch agreement of 95%.

Cortisol concentrations

We used values from T1 (i.e., sample taken at 21:30 h), T2 (00:00 h), T3 (06:00 h), and T4 (06:30 h) to derive another three outcome variables: change in cortisol concentration during the first half of the night ($\Delta\text{CortFirst}$; T2–T1); change during the second half of the night ($\Delta\text{CortSecond}$; T3–T2) and cortisol awakening response (CAR; T4–T3).

Power analysis and sample selection

A priori power analyses suggested the sample size be set at $N=90$ (45 per group) to achieve statistical power of

at least 0.95 using a cross-sectional matched-participant design investigating between-group differences (Cohen's $d=0.70$; $\alpha=0.05$) (35). We chose a medium-to-large effect size parameter because the only published investigation of baseline sleep architecture in AD patients reported an average Cohen's d of 0.72 across all measured sleep variables (15). However, given the rarity of AD, both globally and in South Africa (there are fewer than 200 patients in the SAAD database), along with our stringent eligibility criteria (e.g., fewer than 50 of the patients in the database met our age-related inclusion criterion), we could only enroll seven patients (and hence $N=14$). This sample size generated statistical power of 0.34.

Descriptive and inferential statistical analyses

We completed all analyses using SPSS version 24 and R version 3.4.3, with the threshold for statistical significance (α) set at 0.05 unless noted otherwise. Given the small N and non-normal distribution of outcome variables, we used a non-parametric statistical test (the Mann–Whitney U test) to conduct between-group comparisons for (a) sociodemographic characteristics, (b) PSQI total score, (c) PSG-measured sleep quality and architecture for both the whole-night and split-night datasets and (d) cortisol-related variables. We conducted a Fisher's exact test to determine the magnitude of association between group (AD vs Control) and proportion of individuals classified as poor sleepers (PSQI total score >5). A series of Wilcoxon signed-rank tests compared subjective and objective measures of total sleep time, WASO, sleep efficiency and sleep latency. Because of our small N , we used scatter plots to describe associations between (a) sleep variables and cortisol concentrations and (b) patient disease characteristics and sleep variables/cortisol concentrations.

Results

Sample characteristics

By design, there were no significant between-group differences in terms of age ($P=0.654$) or education ($P=0.165$; Table 1). Analyses also detected no significant between-group BMI differences ($P=0.257$).

Patient clinical characteristics

Two patients had hypothyroidism and one had diabetes. Structured psychiatric interviews suggested one could be

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Table 1 Overall sample characteristics ($n=14$), and patient clinical characteristics ($n=7$).

Variable	Addison's disease ($n=7$)	Healthy controls ($n=7$)	<i>U</i>	<i>P</i>	ESE
Age					
Median (IQR)	40 (23–44)	44 (21–50)	21	0.654	0.12
Range	20–55	20–50			
Education					
Median (IQR)	15 (12–16)	12 (12–15)	14.5	0.165	0.37
Range	12–18	12–15			
Body mass index ^a					
Median (IQR)	25.64 (21.13–34.89) ^e	23.24 (20.20–25.43)	8	0.257	0.34
Range	20.05–37.55	19.26–26.37			
Age at diagnosis (years) ^b					
Mean (S.D.)	25.83 (16.27)	–	–	–	–
Range	7–54				
Duration of AD (years) ^b					
Mean (S.D.)	10.00 (4.82)	–	–	–	–
Range	1–14				
Total hydrocortisone dose (mg)					
Mean (S.D.)	23.57 (6.90)	–	–	–	–
Range	15–35				
Hydrocortisone (mg/kg) ^c					
Mean (S.D.)	0.30 (0.10)	–	–	–	–
Range	0.18–0.46				
Number of doses per day					
Mean (S.D.)	2.29 (0.49)	–	–	–	–
Range	2–3				
Fludrocortisone dose (mg) ^d					
Mean (S.D.)	0.14 (0.38)	–	–	–	–
Range	0.10–0.50				

ESE, effect size estimate (in this case, r for Mann-Whitney U tests).

^aCalculated by dividing the participant's weight by height² (information obtained from the sociodemographic questionnaire); ^bData based on 6 patients (1 patient did not provide the requisite information); ^cData based on 6 patients (1 patient did not provide weight-related information); ^dData based on 5 patients (2 patients were not prescribed this medication); ^eData based on 4 participants (2 patients did not provide details of their height, and 1 did not provide details of her weight).

diagnosed with generalized anxiety disorder, agoraphobia and current manic episodes, and another two with generalized anxiety disorder. Tables 1 and 2 present further details regarding these characteristics, as well as individual regimens of immediate-release hydrocortisone.

Between-group comparisons: subjective sleep data

A larger proportion of patients than controls were classified as poor sleepers: four patients (57.1%) and two controls (28.6%) self-reported a PSQI total score >5 , $P=0.296$, $V=0.29$. On average, patients reported poorer sleep quality (i.e., obtained higher PSQI total scores) than controls: AD: Median (IQR)=6 (3–8) vs Control: Median (IQR)=3 (2–6), $U=15.5$, $P=0.242$, $r=0.31$.

Between-group comparisons: objective sleep data

Several extreme outliers (>3 s.d.s from the mean) were present in the data (seven related to sleep outcome

variables, two to cortisol outcome variables and one to patient timing of last daily dose; Supplementary Fig. 1, see section on [supplementary data](#) given at the end of this article). Results of analyses described below are reported with and without outliers.

Both patients and controls displayed the typical cyclical nature of sleep, with more SWS during the first half of the night and more REM sleep during the second half of the night. Of note is that the patient who took

Table 2 Patient medication regimen ($n=7$).

Patient number	Total dose (mg)	Dose 1 (time, h)	Dose 2 (time, h)	Dose 3 (time, h)
1	15	10 (08:00)	5 (14:00)	–
2	20	10 (06:30)	5 (11:30)	5 (16:00)
3	20	15 (06:00)	5 (17:00)	–
4	20	10 (07:00)	10 (21:00)	–
5	25	15 (06:00)	5 (12:00)	5 (16:00)
6	30	20 (07:00)	10 (16:00)	–
7	35	25 (07:00)	10 (16:00)	–

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Table 3 Objective sleep quality and architecture in patients and controls ($n=14$). Data are presented as median (IQR).

Variable	Addison's disease ($n=7$)	Healthy controls ($n=7$)	U	P	ESE
Whole night					
Sleep efficiency	82.52 (81.9–87.15)	83.47 (79.37–87.4)	23	0.424	0.01
Sleep latency	20.85 (8.35–26.9)	15.85 (10.35–32.35)	22	0.375	0.09
REM latency	51.35 (49.85–96.82)	142.82 (82.35–206.35)	11	0.042*	0.46
WASO	57.73 (37.85–65.35)	65.85 (51.35–72.35)	17	0.169	0.26
N1 time	52.98 (44.85–59.85)	55.98 (45.85–66.85)	20	0.283	0.15
N2 time	247.35 (212.85–252.85)	185.32 (163.35–206.85)	4	0.005*	0.70
SWS time	48.85 (44.35–52.85)	75.82 (56.35–93.35)	1.5	0.002**	0.79
REM time	81.48 (61.85–99.85)	92.35 (55.35–108.35)	20.5	0.305	0.14
N1%	13.5 (10.65–14.45)	14.85 (11.25–18.15)	17	0.169	0.26
N2%	57.77 (50.65–61.25)	47.35 (40.15–49.15)	3	0.004*	0.73
N3%	12.15 (10.85–12.75)	19.55 (13.75–20.45)	0.5	0.001**	0.82
REM%	20.45 (14.35–23.15)	22.42 (14.95–23.85)	20	0.282	0.15
Awakenings	9.07 (5.85–10.85)	8.85 (6.85–10.07)	21.5	0.350	0.10
First half of the night					
Sleep efficiency	79.5 (77.5–83.9)	76.2 (70.9–83.6)	15.5	0.125	0.31
WASO	26.4 (22.9–30.9)	29.9 (23.9–43.9)	17.5	0.185	0.24
N1 time	22.9 (19.4–32.4)	25.4 (19.4–27.9)	22	0.375	0.09
N2 time	122.9 (104.9–128.4)	80.9 (73.4–94.9)	6	0.009*	0.63
SWS time	33.4 (15.4–43.4)	55.7 (52.9–57.4)	< 0.1	0.001**	0.84
REM time	26.9 (12.9–29.4)	19.4 (16.9–31.4)	22	0.375	0.09
N1%	12 (9.4–16.6)	14.3 (13–15.9)	20	0.283	0.15
N2%	61.4 (52.9–62.9)	44.9 (39.7–45.7)	4	0.005*	0.70
N3%	18.5 (7.5–22.9)	28.6 (27–33.1)	< 0.1	0.001**	0.89
REM%	12.8 (9–13.5)	11.9 (9.3–17)	24	0.475	0.02
Awakenings	5.2 (2.9–5.9)	4.9 (3.9–5.7)	247	0.474	0.02
Second half of the night					
Sleep efficiency	89.1 (81.2–94.8)	90.6 (82.84–95.86)	21	0.328	0.12
WASO	30.5 (11.9–45.4)	25.7 (9.85–40.85)	20	0.283	0.15
N1 time	27.9 (21.9–31.4)	31.5 (18.85–42.35)	22	0.375	0.09
N2 time	120.9 (109.9–121.9)	111.35 (87.85–115.35)	12	0.055	0.43
SWS time	10.4 (8.4–15.4)	21.1 (0–35.85)	21	0.327	0.12
REM time	58.1 (32.4–75.4)	64.5 (38.35–84.5)	19	0.241	0.19
N1%	12.6 (9.6–13.9)	15.3 (7.9–20.4)	22	0.375	0.09
N2%	55.3 (51.6–61)	49 (36.8–55.6)	12	0.055	0.43
N3%	4.9 (3.7–6.9)	9.8 (0–14.9)	20	0.283	0.15
REM%	26.7 (16.3–32)	29.9 (18.5–35.5)	18	0.203	0.22
Awakenings	4.7 (2.9–4.9)	2.85 (1.85–4.85)	17	0.167	0.26

* $P<0.05$; ** $P<0.004$ (statistically significant after the Bonferroni correction).
ESE, effect size estimate (in this case, r); WASO, wake after sleep onset.

her last hydrocortisone dose latest in the day had the longest sleep latency (taking over an hour to fall asleep) and the shortest REM latency (less than 50min). For all participants, the brief awakenings at T2 had no detectable effect on sleep EEG. Results of between-group analyses are depicted in Table 3.

Across the whole night (Fig. 2) and during the first half of the night, patients with AD experienced, on average, significantly less SWS than controls. Analyses detected no significant between-group differences for measures pertaining to the second half of the night (all P s > 0.055). This pattern of results remained almost identical when data sets containing extreme outliers were removed from the analysis. The one exception here was that, in the

outlier-free analysis, patients with AD had significantly more N2 sleep during the first half of the night than controls.

Within both groups, subjective sleep reports (for past-month sleep) were consistent with objective PSG-measured sleep on the experimental night (i.e., the analyses detected no significant between-measure differences; Table 4).

Between-group comparisons: cortisol concentrations

Analyses detected significant between-group differences at T4 (patients had significantly lower cortisol levels 30 min post awakening) and for CAR (Table 5). Of note regarding the latter is that five of the six control participants for

Figure 2

Distribution of sleep stages across the whole night, for each patient and control.

whom the variable could be calculated experienced a positive CAR, whereas none of the patients did. Furthermore, whereas controls experienced an increase in cortisol levels during the second half of the night, patients experienced a decrease. This pattern of results held when extreme outliers were removed from the data.

The pattern of cortisol concentrations was relatively homogenous for controls, but more variable for patients (Fig. 3). Of note is that, compared to all other patients, patient 4 (i.e., the individual who took her last

hydrocortisone dose latest in the day) had a substantially higher cortisol concentration at bedtime and experienced the biggest decline in concentrations during the second half of the night.

Correlations: sleep and cortisol

We described associations, within each group separately, between sleep in each half of the night and specific cortisol values. Because three controls had missing cortisol values at midnight, we do not describe relationships of sleep outcomes with cortisol values at midnight, or with $\Delta\text{CortFirst}$ and $\Delta\text{CortSecond}$, in this group.

Sleep during the first half of the night and cortisol concentrations

Here, we describe associations between PSG outcome variables, taken during the first half of the night, and three cortisol outcome variables: (a) the measure taken at bedtime, (b) the measure taken at midnight and (c) $\Delta\text{CortFirst}$. Within the AD group, patients with higher cortisol concentrations at bedtime had fewer awakenings, and patients with higher cortisol levels at midnight had less SWS. When outliers were removed, only the association between cortisol levels at midnight and SWS remained.

Within the healthy control group, those with higher cortisol concentrations at bedtime had shorter REM latencies, better sleep efficiency and less SWS. Generally, this pattern of results held when outliers were removed; the only exception was that cortisol levels at bedtime and sleep efficiency were no longer associated.

Sleep during the second half of the night and cortisol concentrations

Here, we described associations between PSG outcome variables, taken during the second half the night, and three cortisol outcome variables: (a) the measure taken

Table 4 Comparison of subjective and objective sleep measures in patients and controls ($n = 14$). Data are presented as median (IQR).

Variable	Addison's disease ($n = 7$)					Control ($n = 7$)				
	PSQI	PSG	z	P	ESE	PSQI	PSG	z	P	ESE
TST (h)	7 (7–7.5)	6.93 (6.71–7.12)	−0.34	.735	0.09	7 (6.5–8)	6.59 (6.37–7.26)	−0.34	.735	0.09
WASO (min)	42 (30–90)	57.73 (37.85–65.35)	−0.85	.398	0.23	35 (0–50)	65.85 (51.35–72.35)	−1.86	.063	0.50
Latency (min)	10 (5–30)	20.85 (8.35–26.90)	−0.68	.499	0.18	10 (4–15)	15.85 (10.35–32.35)	−1.86	.063	0.50
Efficiency (%)	90.32 (70–96.77)	82.52 (81.90–87.15)	−0.68	.499	0.18	86.67 (80–89)	83.47 (79.37–87.40)	−0.68	.499	0.18

ESE, effect size estimate (in this case, r for Wilcoxon signed-rank tests); TST, total sleep time; WASO, wake after sleep onset.

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Table 5 Cortisol concentrations in patients and controls ($n=14$). Data are presented as median (IQR).

Variable	Addison's disease ($n=7$)	Healthy controls ($n=7$)	<i>U</i>	<i>P</i>	ESE
T1 (21:30 h)	8.02 (5.91–10.29)	5.28 (4.58–6.26) ^b	5	0.011*	0.64
T2 (00:00 h)	13.98 (11.01–20.29)	7.30 (6.67–8.73) ^c	1	0.007*	0.74
T3 (06:00 h)	12.62 (7.22–16.14) ^a	12.32 (10.99–20.24) ^d	12	0.169	0.28
T4 (06:30 h)	6.70 (4.32–10.41)	21.67 (14.37–25.58)	4	0.002**	0.77
Δ CortFirst	5.49 (1.37–11.92)	2.41 (0.74–4.15) ^c	7	0.093	0.40
Δ CortSecond	–1.45 (–6.27 to 2.90) ^a	5.50 (3.71–7.53) ^c	1	0.009*	0.74
CAR	–5.00 (–8.75 to –2.50)	3.50 (2.00–8.25) ^d	0.5	0.003**	0.78

^aData based on six participants (1 sample returned as insufficient for analysis); ^bdata based on six participants (1 sample returned as insufficient for analysis); ^cdata based on four participants (2 samples returned as insufficient for analysis; one sample was not obtained due to researcher error); ^ddata based on six participants (1 sample returned as insufficient for analysis); * $P<0.05$; ** $P<0.007$ (statistically significant after the Bonferroni correction). CAR, cortisol awakening response; ESE, effect size estimate (in this case, r for Mann–Whitney U tests).

at midnight, (b) the measure taken upon awakening and (c) Δ CortSecond. Within the AD group, patients with higher cortisol concentrations at waking had better sleep efficiency and those who had larger decreases during the second half of the night experienced more SWS. When outliers were removed, only the association between cortisol levels upon awakening and sleep efficiency remained.

Within the healthy control group, those with higher cortisol concentrations at waking had more REM sleep. This association remained after outliers were removed.

Correlations: patient disease characteristics, whole-night sleep and cortisol

Because nearly all patients took two doses of hydrocortisone medication per day (bar two patients who took three doses/day), we did not examine the relationships between number of doses and sleep/cortisol variables. Patients with a higher total hydrocortisone dose had poorer sleep efficiency and those with a higher dose/kg had longer sleep latencies and less N2%. Patients who took their last dose of medication later in the day had less N3%, higher cortisol levels at bedtime and a larger decrease in cortisol during the second half of the night. When outliers were removed, only the association between dose/kg and N2% remained, however.

Duration of AD appeared to bear no substantial association to any sleep or cortisol outcome variable.

Discussion

Several studies document self-reported disturbances of sleep quality in patients with AD, but few have used polysomnographic measures to confirm these disruptions. The current study aimed to quantify sleep quality and

architecture of patients with AD and to explore the role of cortisol concentrations in altered sleep patterns. Our patient group exhibited altered sleep architecture (specifically, reduced SWS), which was associated with elevated night-time cortisol concentrations.

Our results are consistent with the only other study in the AD literature that reports on PSG-measured sleep when replacement medication is administered as usual (15). Across the two studies, patients spent similar proportions of time in Stage 2 sleep (63% in the current study vs 57% in Gillin *et al.* (15)), in SWS (14% vs 11%) and in REM sleep (19% vs 20%). Moreover, in that study, as in ours, patients exhibited similar sleep quality and architecture to healthy controls, aside from SWS disruptions. As noted earlier, however, Gillin *et al.* (15) did not include matched samples of patients and controls.

SWS plays a critical role in the general physiological restorative function of sleep and in specific cognitive processes such as memory consolidation (36, 37). Hence, disrupted sleep and altered circadian rhythms lead to energy imbalances, fatigue, memory deficits and poorer quality of life (38, 39). Therefore, it is likely that when patients with AD experience decreased SWS they will also experience these negative health outcomes. Indeed, fatigue and memory deficits are features of adrenal failure that persist despite replacement therapy and are major contributors to self-reported impaired health in AD (19, 40).

Regarding between-group differences in night-time cortisol concentrations, patients had higher cortisol levels prior to sleep onset and during the first half of the night, but cortisol deficiencies during the second half of the night. The most marked between-group difference occurred after waking: Whereas most controls experienced a positive CAR, no patient did. These data are consistent with previous reports suggesting that, despite GC replacement therapy, patients with AD experience

Figure 3

Changes in cortisol levels across the night and morning for each patient (A) and control (B). Data for one patient and three controls were not available at various time points – their graphs have dashed lines representing the potential trend in cortisol level changes (except for Control 2 who is missing the first two cortisol measurements and therefore a trend estimation cannot be made).

alterations of normal cortisol diurnal rhythms (41). Moreover, the observed trends during the latter part of the night and early morning are consistent with previous literature reporting on cortisol secretory action in patients with AD (10). Because cortisol plays a vital role in ensuring smooth transitions between sleep stages (42), irregular patterns of secretion in patients with AD might explain their altered sleep architecture.

Consistent with this conjecture, between-group differences in sleep architecture and in cortisol concentrations both occurred during the first half of the night, and our scatter plot analyses confirmed associations between levels of the hormone and discrete sleep parameters. For instance, within the AD group, higher cortisol concentrations at midnight were associated with less SWS, whereas lower cortisol concentrations during the second half of the night were associated with more SWS. These results are consistent with a substantial body

of research indicating that high cortisol concentrations during the first half of the night (as exhibited by our patient group) are associated with less SWS (also exhibited by our patient group; i.e., lower cortisol levels facilitate the initiation and maintenance of SWS) (2, 15). Similarly, controls who had higher cortisol concentrations at bedtime had less SWS. Within the control group, higher cortisol concentrations at bedtime were associated with shorter REM latency, a finding consistent with research indicating that higher cortisol levels facilitate entry into REM sleep (8, 14, 43).

In the current sample, some disease and medication characteristics of patients with AD were associated with sleep parameters and night-time cortisol secretion. Patients who took larger doses of hydrocortisone had poorer sleep efficiency, shorter sleep latency and less Stage 2 sleep. This result is understandable given that larger doses should translate into higher cortisol concentrations, and high cortisol concentrations increase both sleep onset latency and number of awakenings after sleep onset (2). Patients who took their last dose of hydrocortisone later in the day had higher cortisol concentrations at bedtime and less SWS, further supporting the notion that low cortisol levels are needed to facilitate the initiation and maintenance of SWS.

Limitations and future directions

Measurement and sample size issues limit the strength of the conclusions we can draw from the current data. The fact that we took only four saliva samples from bedtime through waking means we are limited in making inferences about how subtle changes in nocturnal cortisol secretory patterns, particularly between midnight and early morning when REM sleep predominates, may influence sleep parameters. Future studies might test the proposition that SWS is more sensitive to cortisol alterations than REM.

Furthermore, although cortisol measurement by saliva has numerous advantages over that by plasma (e.g., stress-free sampling, lower costs, and non-invasive collection methods) (44), salivary cortisol measured by radioimmunoassay has intrinsic problems of variability. In the current sample, for instance, concentrations may have been influenced by contamination of saliva with hydrocortisone tablets (and may explain, for instance, why patients experienced increasing cortisol concentrations between 21:30 h and midnight, despite taking their final medication dose several hours prior to bedtime and the relatively short half-life of hydrocortisone). Given this

uncertainty surrounding radioimmunoassay evaluation of salivary cortisol, future analyses should use liquid chromatography tandem mass spectrometry. Given the unusual finding that cortisol concentrations increased from bedtime to midnight in both patients and controls, further studies are needed to confirm the associations we found between sleep architecture and cortisol levels.

Furthermore, the fact that we gathered sleep data in a laboratory setting, over one night, means the sleep quality/architecture we analyzed may not accurately reflect participants' normal home-based sleep patterns (45). Although our analyses comparing subjective to objective sleep measures suggested that the patterns we captured in the laboratory were similar to those participants reported experiencing at home, future research in the field might take polysomnographic measures of sleep in a more naturalistic environment and over more nights.

Regarding sample size issues, our small *N* meant that, for instance, we could not investigate the influence of biological sex on sleep quality/architecture in patients with AD or the influence of certain disease characteristics on sleep and cortisol measures. It should be noted that time of last dose was relatively homogenous (6 of the seven patients took their last dose between 14:00 and 17:00h), and only two patients had any medical and psychiatric comorbidities. Therefore, any potential investigation of these variables was limited by the characteristics of the current sample.

A fruitful direction for future research might be to experimentally manipulate medication dosage and/or timing in patients with AD and to take repeated measures with each manipulation, thus allowing each patient to act as his/her own control. This design would allow for a true experimental test of the hypothesis that altered cortisol levels impact sleep architecture and would help control for duration of AD diagnosis in individual patients. Similarly, future research might investigate whether modified-release hydrocortisone (which results in cortisol circadian rhythms that are more similar to those presents in healthy adults) improves sleep in patients with AD.

Summary and conclusion

Relative to healthy controls, patients with AD who were on standard hydrocortisone replacement therapy exhibited altered cortisol diurnal rhythms, accompanied by decreases in SWS. Because SWS plays a critical role in the general physiological restorative function of sleep and in memory consolidation, disrupted sleep in patients

with AD may help explain why patients often report and experience fatigue, reduced vitality, poorer health and memory impairments. Hence, interventions focused on treating sleep disruptions in patients with AD might be especially helpful in improving specific aspects of their cognitive functioning and their overall quality of life.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/EJE-18-0439>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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Author contribution statement

M H and K G F T designed the study; M H collected the data; M H and K G F T analyzed the data and interpreted the results; all authors prepared the manuscript.

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