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Sleep-independent circadian rhythm of aldosterone secretion in healthy young adults

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ABSTRACT

Objective: A diurnal variation in urine output has been described in humans, whereby it is lowest at night. Fluid balance hormones such as vasopressin and aldosterone as well as urine output have a diurnal variation. Although the diurnal variation of vasopressin results in part from a circadian rhythm, the variation in aldosterone has until recently been reported to be due to the sleep/wake cycle. The present study used a specialized protocol to explore whether aldosterone has an underlying circadian rhythm.
Methods: Ten healthy participants (average age 23.1) were enrolled in the 57.3-hour protocol that included an 8-hour baseline sleep episode, 40 hours in constant routine conditions (wakefulness, food and fluid intake, posture, and dim light), and a 9.3-hour recovery sleep. Blood samples for aldosterone were taken every 4 hours. Cosinor analysis was performed on the constant routine data to test the effect of the sleep/wake cycle on overall aldosterone secretion.
Results: There was a significant circadian rhythm during the 40-hour constant routine, independent of sleep, with aldosterone higher at the end of the biological night and lower at the end of the biological day. When analyzing data from the entire 57.3-hour protocol and controlling for this circadian rhythm, aldosterone concentration was significantly higher during the recovery night following the 40-hour sleep deprivation compared to the night spent awake.
Conclusion: We found a significant endogenous circadian rhythm in the secretion of aldosterone, independent of sleep. In addition, as shown previously, there was a significant effect of the sleep/wake cycle on aldosterone secretion.
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Introduction

A diurnal variation in urine output, sodium, and potassium urinary excretion has been described in humans.[1,2] Across the 24-hour day, urine output increases during wake and decreases during sleep both in children[3] and adults.[1,2,4] This rhythm persists even when posture or the timing of the sleep-wake cycle changes, suggesting that there may be an endogenous rhythm of urine production/electrolyte secretion.[4] Urine production and electrolyte secretion/reabsorption by the kidney are governed by the interaction between several fluid/electrolyte balance hormones, including vasopressin/antidiuretic hormone, which controls pure water balance, and the renin-angiotensin-aldosterone-system and atrial natriuretic peptide, which both regulate sodium/potassium balance.

It has been clearly established that vasopressin has an underlying circadian rhythm that is independent of day-night changes in sleep and/or posture in children and adults.[3,5,6] Prior studies had demonstrated a diurnal rhythm of aldosterone,[7–11] which appeared to be sleep-dependent.[12,13] In a study by Charloux et al,[13] participants were kept awake all night and this total sleep deprivation blunted the normal nighttime plasma renin activity and aldosterone increase. When the participants were allowed to then sleep during the following biological day, there was an increase in aldosterone together with that of plasma renin activity. The authors concluded that the typical nighttime increase in aldosterone is strongly influenced by sleep. However, the recent study by Thosar et al[14] suggests that aldosterone also follows an endogenous sleep-independent, posture-independent circadian rhythm of secretion. Confirming the presence of a circadian rhythm of aldosterone could

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open the way to new treatments based on circadian interventions (light therapy, melatonin) for pathologies such as age-related nocturia or shiftwork-induced hypertension.

In the present study, we investigated whether the plasma concentration of aldosterone varies between the biological day and the biological night independent of changes in posture, sleep, and food/fluid intake. We hypothesized that aldosterone was under both a circadian and sleep influence.

Participants and methods

Study participants

Healthy volunteers aged 18-30 years were recruited to participate in a circadian rhythm study at Brigham and Women's Hospital in Boston, MA. The original purpose of the study was to examine the phase and period of circadian rhythms in healthy young early birds and night owls. As part of the study design, we collected extra blood at predetermined sampling times for secondary analyses of hormones involved in fluid and electrolyte balance. Thus, the study was not prospectively designed or powered to investigate whether aldosterone has a circadian rhythm.

Clinical and psychological examinations ensured they were free from any medical or psychiatric disorders. Specifically, participants had to have a systolic blood pressure less than 140 mmHg and a diastolic blood pressure less than 80 mmHg, a body mass index between 19 and 29 kg/m^2, normal blood chemistries, normal blood counts, and a normal electrocardiogram. Those criteria were ascertained during a physical examination.

The protocol, which conformed to the Declaration of Helsinki, was approved by the Human Research Committee at Partners HealthCare System. Each participant gave written informed consent prior to the study.

Study design

For the 2 weeks prior to the laboratory part of the study, participants were asked to keep regular bedtimes and wake times, 8 hours apart, at times of their own choosing. Adherence to the schedule was verified through voicemail time stamp, sleep logs, and activity data from a wrist activity monitor. In-study wake times and bedtimes were calculated using the average sleep timing from the week immediately prior to the study for each participant.

Laboratory study

Participants were studied individually in a private room in the Brigham and Women's Hospital Center for Clinical Investigation that did not contain information about time of day (no windows, clocks, internet, television, etc). Each study began with three baseline days during which the participant was required to remain awake for 16 hours and free to carry out sedentary activities (reading, crafts, listening to music, watching videos) in their room. Lighting was at a typical indoor level during the 16 hours awake (~90 lux) and all lights were turned off for the 8-hour sleep episodes. On day 1, an intravenous (IV) catheter was inserted and we began infusing 1 L of 0.45% saline per day.

On day 4, the participant was awakened at their usual wake time and they began a 40-hour "constant routine" (CR) designed to assess the presence of endogenous circadian rhythms.[15,16] During this protocol, the participant remained awake sitting in bed with the head of the bed at approximately 45°, room temperature was kept constant, lighting was dim (< 3 lux), and they received identical equicaloric hourly snacks that provided 150 mEq Na+/100 mEq K+ (±20%) and 2500 mL fluids per 24 hours, in addition to the 0.45% saline/24 hours infused through the IV line. The participants were required to consume all of each hourly snack, which consisted of a small sandwich, juice, and water. In order to ensure the protocol was maintained, a trained laboratory technician remained in the room with the participant to ensure they remained awake, relatively inactive, did not change posture, and consumed each snack. During those 40 hours, the participants used a urinal or a bedpan while remaining in bed so that they would not change their posture. The 40-hour CR ended at the participant's usual bedtime, at which time the head of the bed was lowered and they were allowed to sleep for 9.3 hours.

Core body temperature measurement and assessment of timing of core body temperature rhythm

Throughout the laboratory study (except for scheduled showers on days 2 and 3), study participants wore a rectal thermistor so that core body temperature data could be collected each minute. Core body temperature data collected during the CR were fitted with a two-harmonic regression curve with first-order autoregressive noise.[17] Data from the first 5 hours and the final 30 minutes of the CR were excluded from analysis due to the masking effects of waking and changing posture at the beginning and end of the CR. The nadir of the fitted curve was used as a circadian phase reference marker, the core body temperature minimum (CBT_{min}).

Hormone sample collection and processing

An IV catheter connected to a 12-foot small-lumen IV line was inserted on day 1 for collection of frequent blood samples. The tubing was passed through a port hole in the wall behind the bed to allow for collection of blood samples from the next room without disturbing the participant's sleep. To keep the IV line patent between samples, 40 mL/h of half normal saline (0.45% NaCl) was infused. This level of salt intake (4.5 g of NaCl/day for 3 days IV) was near the levels used for salt loading protocols (2 days of 0.9% saline or 3 days of 9 g per day of salt as described in[18,19]), to prevent aldosterone bursts caused by low natremia/blood pressure. Blood samples for aldosterone assessment were collected every 4 hours beginning 1 hour after bedtime on night 3 (the baseline sleep episode preceding the CR) and ending 1 hour before wake time of the sleep episode following CR (recovery sleep episode). Each blood sample was immediately centrifuged after collection and the plasma was frozen and kept at − 20°C until assay.

Aldosterone assay

All samples were assayed for aldosterone in duplicate using a radioimmunometric assay using the Coat-A-Count procedure (Siemens Medical Solutions Diagnostics). The sensitivity of this method was 11 ng dL^{-1}, the intra-assay precision was 3.3%, and the interassay precision was 8.4%. Assays were carried out in 2010, approximately 1-2 years after the samples were collected.

Data analysis

To test for the presence of an endogenous circadian rhythm, the aldosterone values from the CR were subjected to cosinor analysis using Proc Mixed in SAS (Statistical Analysis System, v.9.4, SAS Institute Inc, Cary, NC).[20] The cosinor method is a periodic regression analysis, which adjusts a cosine function to the aldosterone values measured at each time point. The rhythm parameters determined are the mesor, which corresponds to the rhythm-adjusted mean; the amplitude, which is the distance from the mesor to the peak of the best-fitting cosine curve; and the acrophase, which refers to the timing of the peak of the best fitting cosine curve. They are calculated by using the estimates of the intercept, the cosine (here, denoted β) and sine (here denoted γ) terms of the model. The intercept

term of the regression model is the mesor. The amplitude is $\sqrt{(\beta^2 + \gamma^2)}$. The acrophase is calculated by taking arctan(-γ/β) (in radians) × period of the rhythm (here assumed to be 24 hours) and if arctan (-γ/β) is negative, adding period.[20]

In addition, we also analyzed the respective contributions of circadian variation and sleep/wake condition to the plasma aldosterone concentration using a mixed model analysis (SAS 9.4) by adding to the cosine and sine terms a sleep/wake condition, which was divided into 3 categories: the baseline sleep episode (normal sleep pressure), wake during the 40-hour CR, and the sleep episode that occurred after the 40 hours of sleep deprivation (high sleep pressure).

Results

Ten young participants, 9 males, 1 female; 19-27 years (23.1 ± 2.81) were enrolled in this study between 2008 and 2009. The average timing of the minimum of their core body temperature rhythm (SD) occurred at 07:09 AM (2:12 hours) while their average wake time occurred at 10:05 AM (1:54 hours).

Using a cosinor analysis within a mixed model (proc mixed with the cosine and sine terms), we found an overall significant circadian variation of plasma aldosterone (cosine term β (SE) = 0.55 (0.17), $p = .002$, sine term γ (SE) = 0.05 (0.19), $p = .791$). The mesor (SE) was 3.36 (0.16) ng mL^{-1} (95%CI = [3.00-3.72]) and the amplitude was 0.56 ng mL^{-1} (95% CI = [0.40-0.99]). The acrophase (timing of highest aldosterone plasma concentration) occurred at 21.8 hours after the CR started (ie, 5.8 hours after habitual bedtime, 2.2 hours before wake time), at the end of the biological night (see Fig. 1), while the lowest plasma concentration occurred 12 hours later in the late biological day. Supplementary Fig. 1 shows each participant's profile of aldosterone plasma concentration during the CR and results of the cosinor analysis at the individual level.

We next analyzed the data including the sleep episodes that occurred before and after the CR. While including the test for rhythmicity by inserting the sine and cosine terms in the model, we also tested for an effect of sleep/wake condition (8-hour baseline sleep [normal sleep pressure] vs. 8-hour night awake during CR vs. 9.3-hour recovery sleep [high sleep pressure]). There was again a significant 24-hour rhythm (cosine term β = 0.85 (0.35), $p = .007$; sine term γ = 0.15 (0.35), $p = .667$). We also found a significant effect of sleep/wake condition ($p = .021$) whereby, at identical circadian times aldosterone plasma concentration was significantly higher during the post-40-hour CR recovery sleep compared to the same hours awake during CR (average (SE) aldosterone concentration difference between baseline night [normal sleep pressure] and wake during CR = 1.38 (0.80) ng dL^{-1}; posthoc Tukey adjusted p value = .224; average (SE) aldosterone concentration difference between recovery night [high sleep pressure] and wake during CR = 2.20 (0.77) ng dL^{-1}, posthoc Tukey adjusted p value $p = .026$; average (SE) aldosterone concentration difference between recovery night and baseline night = 0.82 (1.00) ng dL^{-1}, posthoc Tukey adjusted p value = .694; see Fig. 2). We did not find a significant interaction between the rhythm and sleep/wake condition (cosine × condition, sine × condition, both $p > .05$).

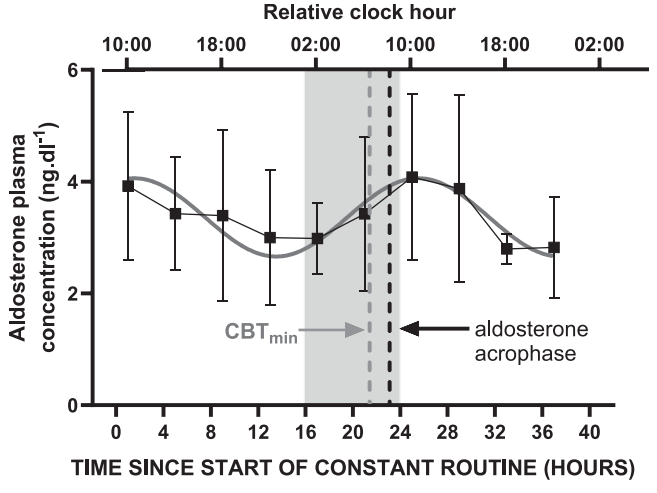

Fig. 1. Mean (± standard deviation) of plasma concentration of aldosterone (in nanograms per deciliter) across the 40-hour constant routine protocol. The start of the constant routine was at wake time (averaging ~10:00 AM as shown on the upper x-axis) of study day 4 and is shown as time = 0 on the lower x-axis. The cosinor model curve is overlaid in dark gray. The habitual sleep episode when participants were kept awake during the constant routine is shown in light gray. CBT_{min} = average core body temperature minimum, another marker of circadian phase, shown in the dashed gray line. CBT, core body temperature

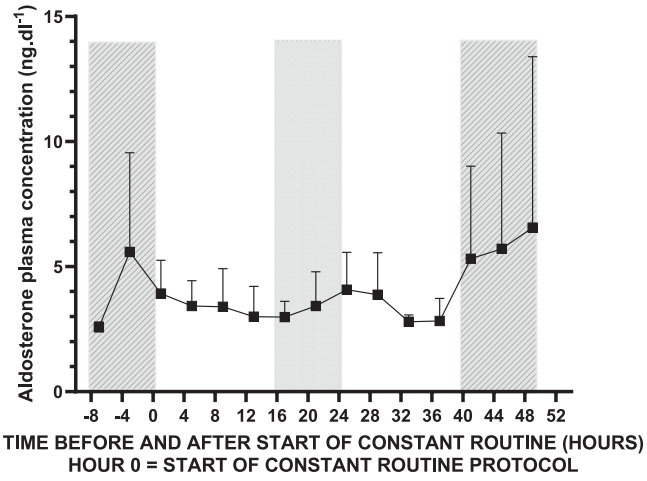

Fig. 2. Mean (± standard deviation) of plasma concentration of aldosterone (in nanograms per deciliter) across the entire 57.3-hour protocol, including (1) a normal sleep pressure 8-hour baseline sleep episode before the start of the constant routine; (2) the 40-hour constant routine; and (3) a high sleep pressure 9.3-hour recovery sleep episode after the constant routine. The start of the constant routine was at wake time of study day 4 and is shown as time = 0. The habitual sleep episode when participants were kept awake during the constant routine is shown in light gray. Hashed gray boxes indicate when the participants slept before and after the constant routine protocol

Discussion

This study confirms the hypothesis that aldosterone shows a sleep-independent endogenous circadian rhythm with a peak of secretion occurring at the end of the biological night/early biological day. Our study thus adds to the study by Thosar et al,[14] which used a forced desynchrony protocol (short light-dark cycles) in middle-aged adults and described a circadian rhythm of aldosterone. In addition, it confirms the influence of sleep itself on aldosterone, with higher aldosterone levels occurring during the biological night when sleep pressure was high. This finding adds to the results from several prior reports,[8,9,11] each of which found a detectable diurnal rhythm of plasma aldosterone with a peak occurring at the end of the biological night/habitual sleep time. In those studies, plasma aldosterone was measured in protocols where young study participants maintained a constant posture, however, received regular breakfast, lunch, and dinner (and no food or fluids during the night), were exposed to a light-dark cycle (with lights out at night), and were allowed to sleep at night. Therefore, day-night differences in food and/or fluid intake, sleep/wake state, activity level, or exposure to light could all have contributed to the detected day/night rhythm in aldosterone. In fact, Charloux et al showed that delta wave activity and aldosterone correlated positively, with aldosterone increasing

20-30 minutes after a slow wave activity burst.[12] As a result, they found that plasma aldosterone concentration increased during sleep, regardless of whether sleep occurred during the habitual biological night or during the biological day.[13]

In our CR protocol, food and fluid intake were distributed evenly throughout a 40-hour interval during which study participants also kept a constant posture and activity level, remained awake, and were exposed to constant levels of dim light throughout the day and night. Even when these potential influences on aldosterone secretion were removed or kept constant across day and night, we found a significant rhythm in aldosterone secretion, confirming our hypothesis that aldosterone secretion is under circadian regulation, with increased secretion during the biological night compared to the biological day. This adds to the known circadian rhythm of another fluid balance hormone, vasopressin, which also reaches a peak during the biological night and a trough during the biological day.[4,5,21,22]

The finding of a circadian rhythm in aldosterone suggested by our study and that by Thosar et al[14] is also consistent with studies that report that urine output and sodium renal excretion follow a circadian rhythm. Mills showed that excretion of sodium and urine output reached their nadir during the biological night.[1,23] Early circadian studies also showed a circadian rhythm in urine production, which, as other variables with a circadian regulation (core body temperature, melatonin, cortisol), could be shifted by exposure to bright light.[24]

In addition to a sleep-independent circadian influence on aldosterone secretion, we found that different nighttime sleep conditions also had a significant independent effect on aldosterone concentrations. Study participants had a higher concentration of aldosterone during the recovery sleep episode, which occurred after the 40-hour sleep deprivation compared to the 8-hour biological night when they were not allowed sleep. This may be due to a direct effect of slow wave activity on aldosterone concentration as described in the study by Charloux et al, where bursts of slow wave activity were significantly associated with aldosterone peaks approximately 30 minutes later.[12] It is widely accepted that there is a substantial increase in slow wave activity during sleep following a 40-hour sleep deprivation compared to a typical 8-hour baseline sleep episode, which may explain why we had the power to detect a difference in aldosterone levels between the night awake and the recovery sleep but not between wake and baseline sleep.[25]

Our findings may have relevance in disorders caused by disturbances in fluid balance, such as nocturia induced by nocturnal polyuria or shift work-related hypertension. Aging is associated with a reduction of the amplitude of some circadian rhythms.[26-28] Two studies investigated the diurnal rhythm of aldosterone secretion in older adults: in one study, aldosterone secretion in healthy older adults was higher and displayed a greater amplitude compared to young adults.[9] In another study, when comparing older men with and without nocturia, no difference in aldosterone levels were found in spite of increased urine output and natriuresis at night found in those with nocturia.[29] Future studies should use the CR protocol to remove all potential confounders so that we can understand whether the endogenous circadian rhythm of aldosterone secretion is dampened in older individuals, particularly in those whose nocturia complaints are independent of a specific urological diagnosis.

Disruption in circadian rhythms induced by shift work has also been associated with increased risks of developing hypertension.[30] A disturbed circadian rhythm of fluid balance hormones such as vasopressin and aldosterone may contribute to the generation of such shift work-related hypertension.

Regulation of the circadian rhythm of aldosterone could be indirectly through substances known to stimulate directly aldosterone secretion, such as the adrenocorticotropic hormone (ACTH) or vasopressin.[31] Adrenocorticotropic hormone and vasopressin have been shown to be under direct circadian regulation, which could then in turn lead to that of aldosterone.[32,33] Interestingly, a diurnal rhythm of expression of the melatonin receptor type 1 has been identified in the adrenal glands of rodents.[34] Pinealectomy has been associated with adrenal hyperplasia and increased aldosterone secretion.[35,36] An effect of melatonin on aldosterone may explain the decrease in blood pressure (without modifications in heart rate) observed in untreated hypertensives given melatonin at night.[37]

It is also likely that aldosterone secretion is under direct molecular clock mechanisms: Cry-null mice, which do not express the clock genes Cryptochrome-1 (Cry1) and Cryptochrome-2 (Cry2), develop salt-sensitive hypertension which is associated with increased synthesis of aldosterone by the adrenal gland.[38]

This study has a few limitations: the sample size was relatively small and we could only sample aldosterone every 4 hours. This would have limited our power to find a difference in aldosterone levels between the night with normal sleep pressure and the night when the participants were kept awake during the CR. This also precluded a fine analysis of the sleep influence on aldosterone. Finally, the pre-CR partial salt loading, meant to avoid bursts of aldosterone caused by lower natremia/blood pressure, may have dampened the amplitude of the circadian rhythm of aldosterone.

In conclusion, we have demonstrated a sleep-independent endogenous circadian rhythm of aldosterone in healthy young adults, confirming a recent study in middle-aged adults using a different experimental paradigm,[14] and adding to the known effects of sleep on aldosterone.[12,13] This circadian contribution to the day-night differences in aldosterone could have implications for better understanding the pathophysiology of fluid-balance disorders such as nocturia and hypertension, particularly in the context of aging and shift work.

Dr. Czeisler's contributions to the work

This project was heavily influenced by the work of Dr. Czeisler in two major ways. First, his development and refinement of the Constant Routine protocol for assessing circadian rhythms in humans made it possible to demonstrate endogenous rhythmicity. The CR achieves this by eliminating periodic changes in behavior (such as postural changes, differences in activity, changes in sleep-wake state) and environment (room temperature, lighting level) and for those things that cannot be eliminated (such as food and fluid intake) it distributes them evenly across day and night. Dr. Czeisler used the CR protocol to demonstrate the endogenous circadian rhythm of several other hormones (cortisol, thyroid stimulating hormone, prolactin, parathyroid hormone[39-42]), inspiring us to investigate whether hormones involved in fluid and electrolyte balance have circadian rhythms that contribute to the observed circadian rhythm in urine production. We are also grateful for Dr. Czeisler's support and mentorship.

Public health relevance

Our finding of a circadian rhythm of aldosterone secretion has relevance for understanding the pathophysiology of fluid-balance disorders such as nocturia and hypertension, particularly in the context of aging and shift work.

Author contributions

The current project is based on a research question proposed by KS. The data come from studies that were designed by JFD and AMC. AMC and JFD conducted the experiments. KS analyzed the data and prepared the figures. KS and JFD wrote the paper. All authors reviewed and approved the paper.

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Declaration of conflicts of interest

Drs. Scheuermaier and Duffy report no conflicts of interest. Dr. Chang reports grants from Kunasan, Inc, and personal fees and nonfinancial support from the University of Miami, both unrelated to this work.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.sleh.2023.10.019.

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