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Diurnal variation of aldosterone and plasma renin activity: timing relation to melatonin and cortisol and consistency after prolonged bed rest

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Hurwitz, Shelley, Richard J. Cohen, and Gordon H. Williams. Diurnal variation of aldosterone and plasma renin activity: timing relation to melatonin and cortisol and consistency after prolonged bed rest. *J Appl Physiol* 96: 1406–1414, 2004. First published December 5, 2003; 10.1152/japplphysiol.00611.2003.—Exposure to prolonged bed rest is known to induce changes in the renin-angiotensin-aldosterone system (RAAS) by way of posture, sodium and potassium balance, and stress, which may have serious consequences for patients. We focused on the diurnal variation of the RAAS by investigating changes in the levels of plasma renin activity (PRA) and aldosterone; for comparison to markers of the intrinsic pacemaker and to stress, we measured melatonin and cortisol. PRA, aldosterone, melatonin, and cortisol were measured hourly in 10 normal subjects with standardized sleep patterns, posture, and diet at baseline and after 11 days of prolonged bed rest conducted under a light-dark cycle. Circadian characteristics of hormone secretion patterns were estimated by multiple harmonic regression with excellent goodness-of-fit measures. Variability in the melatonin and cortisol patterns across subjects was minimal. Even for pulsatile hormones, this technique successfully estimated the acrophase, which was the salient feature. Baseline hormone peak times started with melatonin near the middle of the sleep period, followed by PRA, then aldosterone, and then cortisol around wake time. Prolonged bed rest did not induce significant changes in any timing characteristic of the secretion patterns. Baseline and prolonged bed rest peak times for melatonin and cortisol and amplitude characteristics for all hormones were highly correlated, indicating consistency within individuals. These data provide strong evidence that prolonged bed rest of 11 days' duration does not disrupt either the timing characteristics of the RAAS or the intrinsic pacemaker.

renin-angiotensin-aldosterone system; circadian rhythms; harmonic regression

DISTURBANCES OF CIRCADIAN rhythms are becoming an increasingly important area of clinical investigation, particularly for hormones involved in volume homeostasis. Several lines of research have shown that alterations in these rhythms may lead to the worsening of a number of clinical syndromes. Disruption of these rhythms may alter the "normal" sodium volume homeostatic mechanisms, thereby contributing to the volume abnormalities associated with clinical conditions. Disturbances of circadian rhythms are also an important area for space and microgravity research (6).

It is well known that posture, sodium and potassium intake, and stress can modify the activity of the renin-angiotensin-aldosterone system (RAAS). However, consensus is lacking concerning the effect of these parameters on the circadian rhythms of components of this system. In contrast, it is expected that cortisol rhythms are modified by stress (for example, Ref. 22) and would generally not be significantly affected by posture, sleep, or intake of sodium or potassium, although the relationship between cortisol and sleep is quite complex (13, 14, 29). Studies seeking to characterize a circadian rhythm for plasma renin activity (PRA) have reported conflicting results. There is evidence that PRA is driven by the sleep-wake cycle and may not be a circadian process (2, 3). The contrary has also been suggested. Because the rhythm persisted in a patient with primary hyperaldosteronism even without sleep, diurnal renin variation may be an endogenous function (21). A diurnal variation in PRA, independent of posture and diet, was detected by a number of investigators (6, 12, 20), and there is evidence that prolonged bed rest may increase PRA (10, 18), but a shift in timing has not been confirmed. Several studies have evaluated the circadian rhythm of plasma aldosterone in patients and normal subjects. Aldosterone's rhythm is influenced by both ACTH and PRA (17, 23) and usually varies diurnally. However, during 17 days of bed rest on a controlled diet, subjects showed no significant day-night variation for aldosterone excretion, unlike the pre-bed rest condition (19).

Many studies have not controlled sodium and potassium intake. Others controlled sodium and potassium but not fluid intake, calcium, calories, or hours of sleep. If posture was controlled, it was only for a limited period. Previous studies of prolonged bed rest did not control for the effect of sleep deprivation on these rhythms, even though these disturbances can disrupt circadian rhythms and modify sleep patterns. Indeed, only a few have linked the rhythms of these hormones to the activity of the markers of the central nervous system's circadian pacemaker.

There is documentation that both melatonin and core body temperature are excellent surrogate markers for the activity of the human endogenous circadian pacemaker (7). Seven days of head-down-tilt bed rest produced no significant change in the timing of the peaks of temperature (24). An earlier study showed that the mechanisms underlying the body temperature rhythm are more closely correlated with the endocrine system

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than with the cardiovascular system (31). Importantly, melatonin and cortisol both exhibit rhythmicity that is relatively unaffected by sleep and meals, an advantage over core body temperature, which declines during sleep (7). The diurnal pattern of a cortisol excretion rhythm persisted through a bed rest period in one study (19). The present study expands our understanding of the circadian rhythms of the components of the RAAS by focusing on timing characteristics and linking those components to the periodicity of other circadian markers. In a group of normal subjects, the circadian rhythms were determined for PRA and aldosterone, hormones that clearly control sodium and volume homeostasis. Measurements of two control substances also were obtained: melatonin, as a surrogate marker for the activities of the endogenous circadian pacemaker, and cortisol, as a hormone that shares a responsiveness to stress with PRA and aldosterone. To remove any influence of variation in sodium, potassium, calcium, or fluid intakes, all subjects studied had a constant intake of these factors. To eliminate the confounding effect of postural change in modifying these rhythms, studies were performed first after subjects were in a supine posture for a single day as the baseline condition. Then, without subjects ever deviating from the baseline posture state, studies were repeated after 11 days of their continuous recumbency to determine whether a prolonged constant supine position modifies the circadian phase relationships among these hormones.

The temporal order of major secretory periods of melatonin, cortisol, PRA, and aldosterone also was investigated. Because melatonin is a centrally mediated marker of the circadian pacemaker, if prolonged bed rest disrupts the secretory pattern of melatonin, disruptions of cortisol, PRA, and aldosterone may follow indirectly because of the disruption of the internal pacemaker or directly because of a causal link between hormones. If changes in levels of aldosterone consistently and closely follow changes in levels of cortisol (as a surrogate for ACTH) or renin, a cause-and-effect link is feasible.

MATERIALS AND METHODS

Subjects. Ten male volunteer nonsmoking subjects, age 25–59 yr, were recruited. Subjects were selected after physical and psychological examination. Screening laboratory tests included electrocardiogram, complete blood count with differential, chemistry profile, thyroid function tests, and urinalysis. The exclusion criteria included hypertension, diabetes, coronary artery disease, renal insufficiency, thyroid disease, viral hepatitis, and anemia. Exclusion criteria also included a history or evidence for psychiatric disorders by the Minnesota Multiphasic Personality Inventory; any alcohol, drug, or medication use as verified by a complete toxicological analysis of blood and urine; and known sleep disorders, shift work, and transmeridian travel within 6 mo before the study. For 1 wk before the study, all subjects kept a log of their sleep and called the clinic on going to bed and arising. Deviations from a 10 PM to 6 AM sleep schedule were not allowed. The protocol was approved by the Brigham and Women's Human Subjects Research Committee, and informed written consent was obtained.

Experimental procedures. The studies were performed in Intensive Physiological Monitoring beds in the Clinical Research Center, Brigham and Women's Hospital. Subjects were placed on an isocaloric diet with 200 meq sodium, 100 meq potassium, 1,000 g calcium, and 2,500 ml fluid intake daily throughout the study. No smoking or caffeine-containing drinks were allowed during the study.

Each protocol began with 5 days of dietary and activity equilibrium. Data collection occurred on the 6th to 7th day and on the 18th to 19th day of the protocol and involved taking 30 hourly blood samples from 7 AM until 12 noon the next day. Samples were later assayed in batch for levels of melatonin, cortisol, aldosterone, and PRA. Lights were off between 10 PM and 6 AM, and subjects were told to sleep for that period. Otherwise, illumination was at 150 lux during wake time, and subjects were allowed to read or listen to recorded music. They were not allowed to nap. Before the baseline condition, subjects were allowed unrestricted ambulation in the room from 6 AM to 10 PM. Subjects were then supine for the day of baseline data collection. During the prolonged bed rest intervention, subjects were strictly confined to bed with their heads tilted 4° down for 11 days. They were allowed to lie on their sides, backs, or stomachs, and they voided and defecated in the supine position. They ate all meals lying on their sides, propped up with one elbow at 6 AM, 12 PM, and 6 PM. The post-bed rest data collection was identical to the baseline supine data collection.

Chemical analysis. Plasma melatonin was measured by an RIA that uses [^{3}H]melatonin (New England Nuclear, Boston, MA) as the tracer (9). Standards were prepared from a fresh pool of charcoal-stripped plasma and unlabeled melatonin (Sigma Chemical, St. Louis, MO). The limit of sensitivity for the assay, twice the SD of maximal binding, was 37.3 pmol/l. The intra-assay coefficient of variation was 14.9% at a mean melatonin value of 82.0 ± 5.0 (SE) pmol/l, 10.0% at a mean melatonin value of 140.0 ± 5.7 pmol/l, and 5.6% at a mean melatonin value of 278.4 ± 6.3 pmol/l. Cortisol and PRA were measured by using a commercial antibody-coated tube RIA kit (Incstar, Stillwater, MN) (8). Plasma was incubated for 30 min at pH 5.5 and 37°C. The method used for aldosterone was the Diagnostic Products Coat-A-Count RIA procedure (Diagnostic Products, Los Angeles, CA). The samples were separated by using a radiolabeled aldosterone-specific antibody immobilized on the wall of a polyethylene tube. Aldosterone was then measured with a gamma counter.

Statistical analysis. The goals of the statistical analysis were to obtain circadian timing estimates for each individual for melatonin, cortisol, aldosterone, and PRA; to compare the post-bed rest condition

Table 1. *Electrolyte and volume status*

	BL			PB			Δ			
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*P*
Weight, kg	10	78.0	9.7	10	77.1	9.9	10	−0.9	0.6	0.001
Urine volume, ml/24 h	10	2,997	612	10	2,364	439	10	−634	643	0.02
Urine Na, meq/24 h	10	238	53	10	170	32	10	−68	48	0.001
Urine K, meq/24 h	10	85.7	13.7	10	85.4	13.3	10	−0.4	14.9	0.94
Serum Na, meq/l	10	139	3.1	10	138	1.4	10	−0.6	3.5	0.61
Serum K, meq/l	10	4.4	0.3	10	4.4	0.4	10	−0.04	0.4	0.81
Hemoglobin, g/dl	9	14.5	0.3	10	14.2	0.6	9	−0.3	0.5	0.11
Hematocrit, %	9	41.4	1.4	10	40.1	2.0	9	−1.2	2.1	0.12

n, No. of subjects. BL, baseline; PB, prolonged bed rest; Δ, PB − BL; Na, sodium; K, potassium.

with the baseline condition; and to investigate the relationships among the estimated times of peak hormone secretion. First, the data were examined for laboratory errors in relation to the half-life of each hormone, and impossible values were deleted. For melatonin, a total of five nonconsecutive values for four subjects and two conditions were excluded, and one subject had no melatonin data after prolonged bed rest. For cortisol, one subject's baseline condition was excluded from analysis because of a gap in the data from 4 PM to 8 PM. For aldosterone, one value was deleted and one subject's baseline condition could not be analyzed because the pattern had no structure, varying only from 2.5 to 2.7 ng/ml. For PRA, two subjects had no apparent structure for both baseline and follow-up, with ranges of 0.7 $ng \cdot ml^{-1} \cdot h^{-1}$ or less. We used the remaining valid data and did not impute any missing data. Plots of the raw data revealed salient asymmetric sinusoidal patterns for all remaining subjects, hormones, and conditions.

The data for individual subjects were fit with three-harmonic nonlinear least squares regression models. This multiple harmonic approach allowed for waveform asymmetry, which was especially evident for melatonin, for which there were long flat periods between secretions. Multiple harmonics also were applicable to other hormones, with secondary peaks of lesser amplitude. The overall period was assumed to be 24 h. With 30 hourly samples, the influence of the early measurements was counterbalanced by stable later measurements. Standard goodness-of-fit statistics were examined in conjunction with graphical goodness of fit (4). These models captured the important structure in the data and provided sufficient precision for obtaining circadian phase estimates. The resulting composite multiple-harmonic fits provided the estimates for all of the phase markers analyzed. The time of the maximum secretion and all amplitude components were analyzed for melatonin, cortisol, aldosterone, and PRA. For melatonin, the onset, offset, area, and duration of the secretion also were estimated from the composite fits and analyzed. The onset and offset of the melatonin secretion were defined as the times the ascending and descending regressions crossed 25% of the maximum. Statistical analyses included paired *t*-tests to evaluate the

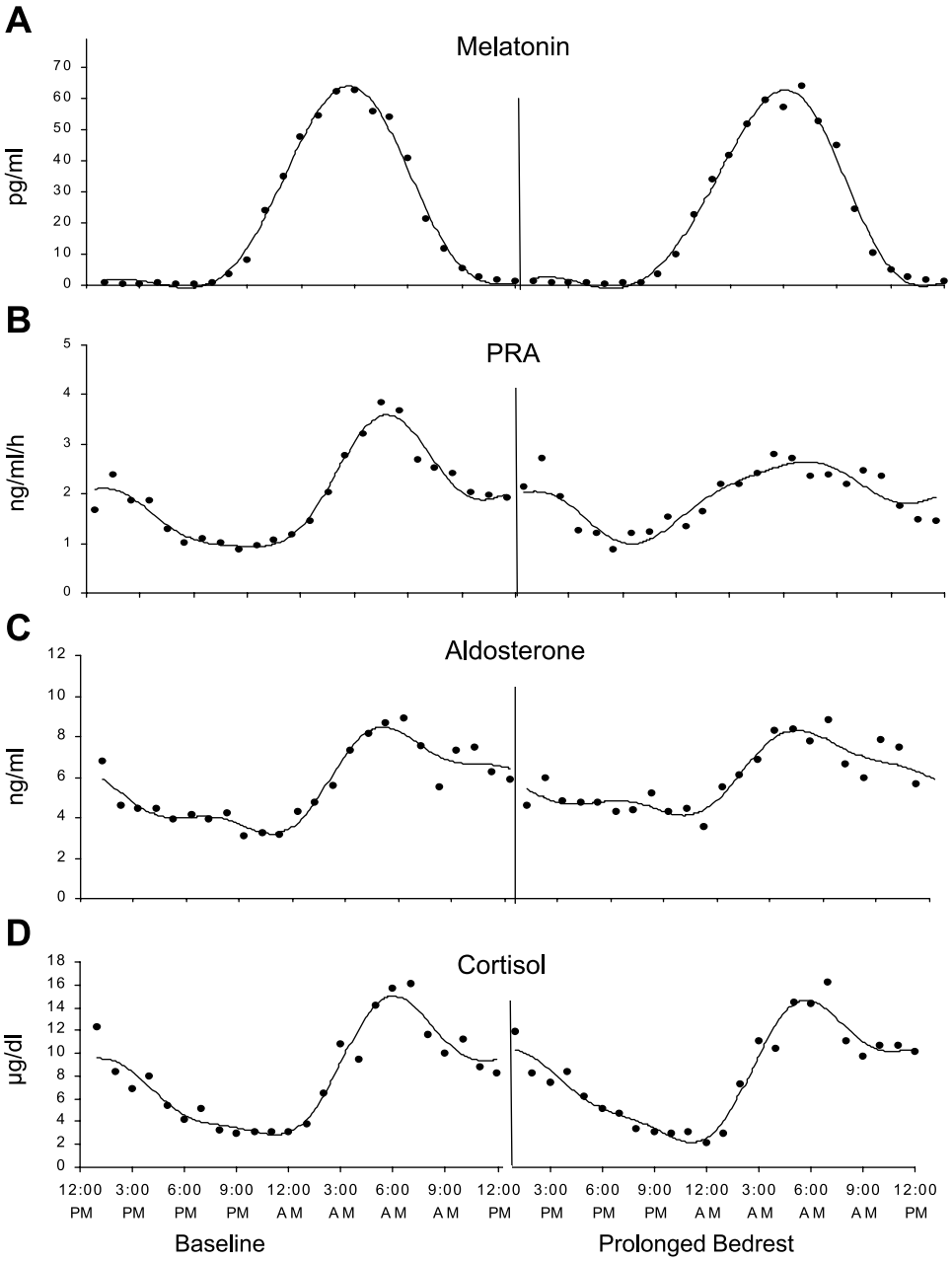

Fig. 1. Patterns of hormone secretion at baseline and after prolonged bed rest. *A*: melatonin. *B*: plasma renin activity (PRA). *C*: aldosterone. *D*: cortisol. Solid lines, average of the harmonic regressions that were fit to individual subject data; ●, group averages of the observed data.

Table 2. *Time of secretion peak (acrophase) for melatonin, cortisol, PRA, and aldosterone*

	BL			PB			Δ				Correlation	
Hormone	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*P*	*r*	*P*
Melatonin	10	2:19 AM	1.33	9	2:35 AM	1.26	9	0.26	0.63	0.26	0.89	0.001
PRA	8	3:57 AM	1.76	8	4:13 AM	2.21	8	0.26	2.14	0.74	0.44	0.27
Aldosterone	9	5:39 AM	1.99	10	4:59 AM	2.28	9	−0.59	3.23	0.60	−0.07	0.86
Cortisol	9	6:28 AM	1.25	10	6:01 AM	1.55	9	−0.34	1.00	0.33	0.78	0.01

n, No. of subjects. PRA, plasma renin activity.

effect on all circadian estimates of the prolonged bed rest intervention and Pearson correlations to assess the concordance of the peak secretion times between the two conditions and among the four hormones. Distributions were inspected for normality and skewness. With sample sizes of 10 or fewer, there is low power to detect departures from normality as well as to reject the main null hypotheses; therefore, effect sizes should be taken into consideration, with *P* values as guides to interpretation. Mean differences and SDs of time estimates were expressed in hours. Peak time orders were analyzed by exact binomial tests to reject the hypothesis of no order in favor of the stated order. A standard power analysis and a standard equivalence analysis were presented for the effect of prolonged bed rest on hormone peak time (15). The post hoc power analysis was valid because the alternative hypotheses were defined without relation to observed data: the simple assertion of 1-, 2-, or 3-h time shifts. The equivalence analysis was valid because the equivalence bounds were preselected to correspond to an external standard unrelated to observed data: the 1-h minimum discrete time lag in transmeridian travel. If the 100(1–2α)% confidence interval fell within the predetermined equivalence range, equivalence was asserted. The SAS version 8.2 statistical package was used.

RESULTS

Table 1 presents electrolyte and volume status at baseline and after prolonged bed rest. As expected, there was a significant decrease in body weight, urine volume, and urine sodium ($P < 0.05$). The urine and serum potassium, serum sodium, hemoglobin, and hematocrit did not change significantly ($P > 0.10$).

Harmonic regressions were produced for each subject and each hormone at baseline and after prolonged bed rest. Figure 1 shows the averaged harmonic regressions for each hormone in each condition superimposed on averaged raw data. Thirty hours of raw data contributed to 24-h circadian fits, with the 7 AM-to-noon predictions derived from the contributions of two measurements each. The regressions accounted for the asymmetry in the waveform, which was particularly pronounced for melatonin, for which the peak was farther from the mesor (midline estimating statistic of rhythm) than was the nadir. The goodness-of-fit measures for each subject in each condition varied according to hormone, depending on amount of pulsatility, with the best fits for melatonin (median $R^2 = 0.95$) and good fits for cortisol (0.78), aldosterone (0.63), and PRA (0.61). Prolonged bed rest did not induce significant changes in R^2 for any hormone ($P > 0.70$). The time of the main secretion peak was realistically estimated by harmonic regression for all hormones (Fig. 1).

Table 2 presents the estimated peak times for melatonin, cortisol, PRA, and aldosterone at baseline and after prolonged bed rest. The estimates were not affected significantly by prolonged bed rest ($P \geq 0.26$). The average change for melatonin was <0.5 h, and there was significant consistency based on the correlation ($r = 0.89$, $P = 0.001$). The mean peak times started with melatonin around 2:20 or 2:30 AM and ended ~4 h later with cortisol at around 6:00 or 6:30 AM. The melatonin secretion was sufficiently discrete to analyze further. Table 3 shows that the intervention had no significant effect on the onset time, offset time, duration, or area of the melatonin secretion ($P \geq 0.26$) and that there was strong consistency across condition ($P \leq 0.01$).

Table 4 presents an analysis of the hormone peak time sequence. There was strong evidence in both conditions to suggest that melatonin precedes aldosterone ($P \leq 0.02$) and that aldosterone precedes cortisol ($P \leq 0.05$), confirming the observed mean sequence presented in Table 2. The PRA peak times were more variable, and thus the ordering for the PRA peak time could not be confirmed. With melatonin considered to be a surrogate measure of the intrinsic circadian pacemaker, Table 5 presents the baseline and prolonged bed rest peak times for cortisol, PRA, and aldosterone expressed in relation to the melatonin peak time instead of the external clock time. The cortisol peak time followed the melatonin peak time by 4.31 ± 0.98 h at baseline and by 3.46 ± 1.45 h after prolonged bed rest. This mean shift of −0.73 h for subjects with data in both conditions indicated that there was a tendency for the cortisol peak to be 44 min closer to the melatonin peak after prolonged bed rest compared with baseline ($P = 0.06$), and the negative shift was consistent across subjects ($r = 0.78$, $P = 0.02$).

Table 6 shows the relationship of the offsets from melatonin to cortisol, PRA, and aldosterone. After prolonged bed rest, the

Table 3. *Melatonin secretion characteristics*

	BL ($n = 10$)		PB ($n = 9$)		Δ ($n = 9$)			Correlation ($n = 9$)	
Characteristic	Mean	SD	Mean	SD	Mean	SD	*P*	*r*	*P*
Onset time	9:28 PM	1.38	9:32 PM	1.57	0.08	0.91	0.80	0.82	0.007
Offset time	7:28 AM	0.82	7:22 AM	0.73	−0.23	0.58	0.26	0.70	0.03
Duration, h	10.01	1.12	9.83	1.29	−0.31	0.82	0.29	0.78	0.01
Secretion area, pg·h/ml	464.9	169.4	454.7	165.03	−7.49	110.7	0.84	0.80	0.01

n, No. of subjects.

Table 4. *Ordering of the peak hormone secretion times*

Peak Time Order	BL		PB	
	% of Subjects	*P*	% of Subjects	*P*
Melatonin precedes PRA	75	0.14	86	0.06
Melatonin precedes aldosterone	100	0.002	89	0.02
Melatonin precedes cortisol	100	0.002	100	0.002
PRA precedes aldosterone	71	0.23	38	0.86
PRA precedes cortisol	86	0.06	75	0.14
Aldosterone precedes cortisol	88	0.04	80	0.05

melatonin-to-PRA interval and the melatonin-to-aldosterone interval were correlated ($r = 0.79$, $P = 0.04$). Subjects with short melatonin-to-PRA intervals also had short melatonin-to-aldosterone intervals, and subjects with long melatonin-to-PRA intervals also had long melatonin-to-aldosterone intervals.

A power analysis can be useful in a study with 10 subjects and negative findings. Table 7 presents a power analysis for detecting theoretically meaningful, externally derived shifts in hormone peak times due to prolonged bed rest. Shifts of 1, 2, and 3 h were asserted to be meaningful.

Observed variances provided sufficient power to detect a 3-h shift in melatonin, cortisol, and PRA and a 2-h shift for melatonin and cortisol. The average time shifts for all hormones in this study were <1 h in either direction, whether clock times or offsets from melatonin peak times were used. Because the effect of prolonged bed rest on hormone peak times appeared to be negligible (Table 2), an equivalence analysis was performed (Table 8). To test equivalence between conditions, an externally derived equivalence range around zero difference, unrelated to observed data, was asserted, and a $100(1–2\alpha)\%$ confidence interval was calculated. If the confidence interval fell within the predetermined equivalence range, equivalence was concluded. The theoretically meaningful equivalence range was asserted to be ± 1 h to correspond to the minimum discrete time lag in transmeridian travel. Two-sided confidence intervals were compared with upper and lower bounds of ± 1 h. Equivalence was asserted for melatonin and cortisol because the confidence intervals were contained within the equivalence range (Table 8). The hormone peak times before and after prolonged bed rest were equivalent. Although the shifts in peak time of PRA and aldosterone were <1 h, the larger variances rendered inconclusive the determination of equivalence. The same conclusions emerged for all hormones when a range of conventional confidence coefficients were used.

The harmonic regression technique for fitting circadian hormone data allowed asymmetry in the waveform, seen in the amplitude analysis in Table 9. For example, melatonin had long quiet periods and strong secretory periods, so the mesor was not equidistant between the peak and the nadir. Prolonged bed rest did not induce significant changes in any component of the amplitude of the waveform. An apparent decrease in PRA secretion between baseline and prolonged bed rest was noted in the mean data and the plots (Fig. 1). Inspection of the data revealed that one subject had unusually high PRA levels at baseline, with a mesor, amplitude, and rise of 6.3, 10.7, and 6.2 $ng \cdot ml^{-} \cdot h^{-1}$. We could find no reason for the extreme data for one subject. Data for other subjects support the conclusion that the baseline and prolonged bed rest conditions were similar for all parameters. Including the extreme data in the analyses produced plots that appeared different but did not produce statistical significance because of the wide variability among subjects. For the changes attributable to prolonged bed rest, the 95% confidence intervals around the mesor, amplitude, and rise (in $ng \cdot ml^{-} \cdot h^{-1}$) were -1.5, 1.4, -3.5, 1.3, and -2.4, 0.6.

The correlations for the amplitude and waveform estimates showed great consistency in melatonin, cortisol, and aldosterone. PRA appeared to be less consistent than that of the other hormones. However, those correlations were stronger when the subject with the unusually high levels of PRA was excluded. The hormone peak was selected for the multiple hormone analysis presented in Table 10. Consistency was generally not detected, although the change that prolonged bed rest induced in melatonin and the change induced in cortisol were correlated ($r = 0.72$, $P = 0.04$).

DISCUSSION

This study was designed to determine whether the constant supine position modifies the diurnal timing characteristics of the melatonin and the RAAS rhythms in normal subjects. Intake of sodium, potassium, calcium, and calories and fluid volume were tightly controlled to remove them as potential confounding variables. To assess whether stress induced by the prolonged bed rest condition could be a confounding variable, cortisol was measured as a specific readout for any induced stress. Importantly, melatonin levels were measured to determine whether the intrinsic circadian pacemaker had been altered by the prolonged bed rest. These studies suggest that prolonged bed rest does not modify either the intrinsic circadian rhythm or the diurnal rhythms of the components of the RAAS even when volume changes. Findings are relevant for clinical conditions as well as space and microgravity.

Meaningful shifts in hormone peak times attributable to 11 days of prolonged bed rest did not emerge in this study, even in the presence of altered volume. The average time shifts for all hormones were <1 h in either direction whether clock times or offsets from melatonin peak times were used. The variation in peak time was smallest for melatonin and cortisol and largest for aldosterone. Consequently, this study had the greatest power to detect meaningful shifts for melatonin and cortisol

Table 5. *Time from melatonin peak to cortisol, PRA, and aldosterone peak*

Offset From Melatonin*	BL			PB			Δ				Correlation	
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*P*	*r*	*P*
PRA	8	1.13	1.49	7	1.77	1.93	7	0.33	2.44	0.73	−0.12	0.79
Aldosterone	9	3.36	2.31	9	2.43	3.63	8	−0.86	3.14	0.46	0.58	0.13
Cortisol	9	4.31	0.98	9	3.46	1.45	8	−0.73	0.94	0.06	0.78	0.02

n, No. of subjects. *Hormone peak time minus melatonin peak time (in h).

Table 6. *Correlation among melatonin offsets within each condition*

	BL			PB		
Offsets From Melatonin	*n*	*r*	*P*	*n*	*r*	*P*
Cortisol with PRA	7	−0.03	0.95	7	−0.68	0.09
Cortisol with aldosterone	8	0.46	0.26	9	0.33	0.39
PRA with aldosterone	7	−0.24	0.61	7	0.79	0.04

n, No. of subjects. *Melatonin offsets calculated as peak time minus melatonin peak time (in h).

Table 8. *Equivalence analysis for shift in hormone peak time (0 ± 1-h bounds)*

	Confidence Coefficient, α			
Hormone	95%, 0.025	90%, 0.05	80%, 0.10	Conclusion
Melatonin	(−0.15, 0.67)	(−0.09, 0.61)	(−0.01, 0.53)	Equivalent
Cortisol	(−0.99, 0.31)	(−0.89, 0.21)	(−0.77, 0.09)	Equivalent
PRA	(−1.22, 1.74)	(−0.98, 1.50)	(−0.71, 1.23)	Inconclusive
Aldosterone	(−2.70, 1.52)	(−2.36, 0.18)	(−1.97, 0.79)	Inconclusive

and the least power to detect those for aldosterone. Certain conditions in the present study may limit its generalizability. Sleep time was standardized, and the 150-lux wake-time lighting would have suppressed melatonin relative to dimmer conditions, possibly masking small changes. Even though the average changes due to prolonged bed rest for melatonin and cortisol were not significantly different from zero, there was a great deal of consistency as reflected in the significant correlations for both hormones. Prolonged bed rest did not disrupt the tendency for subjects to have relatively early or late peaks. The onset, offset, duration, and area of melatonin secretion were similarly undisturbed by prolonged bed rest.

Under controlled conditions of sleep, posture, and diet, the main secretory periods occurred between 2:15 AM and 6:30 AM, with the order being first melatonin, second PRA, third aldosterone, and last cortisol. It has been reported that, under constant routine conditions, the melatonin peak is near the midpoint of the regular sleep episode (26). In the present study, without constant routine conditions, the result was the same in both conditions, suggesting that this feature of the melatonin secretory pattern result is robust. The time of the melatonin peak correlates with the time of the core body temperature nadir and can serve as an indicator of the intrinsic circadian rhythm. Because 11 days of prolonged bed rest did not disrupt the melatonin secretory pattern, it most likely does not disrupt the core body temperature timing pattern, although the temperature amplitudes during prolonged bed rest may be damped (25, 31). A formal study would be necessary for confirmation.

The analysis of the ordering of the peak secretion times extends previous research reporting significant correlations between hormone levels within subjects and concluding synchronicity, particularly for aldosterone and cortisol (17, 30). However, a significant correlation of levels does not preclude a consistent time lag. The harmonic regression methodology in the present study showed that the hormones are not necessarily synchronous, with aldosterone generally preceding cortisol and melatonin generally preceding aldosterone.

The lack of influence on cortisol by prolonged bed rest confirms previous findings (6) that neither the average levels nor the diurnal rhythm of plasma cortisol are influenced by bed rest. Also, in a more recent bed rest study that included a head-down tilt, the ambulatory cortisol excretion rhythm persisted through the bed rest period (19). Other researchers found a reduction in the cortisol rhythm amplitude with prolonged bed rest and reported that exercised subjects tended to have earlier cortisol peaks, although the sample sizes were small (28). It has been suggested that prolonged bed rest is stressful and that the reduction in body temperature that prolonged bed rest may induce is a common result of stress (31). It may also be a component of the dysregulation seen in circadian misalignment situations such as shift work (27) and transmeridian travel in older pilots (1). One study did not find a reduction in body temperature with prolonged bed rest, but the subjects were seated for part of the experimental time (24). The temperature acrophases were not altered. The additional stress related to exercise and/or slight head-down tilt, in addition to posture changes, may account for differences among previous studies.

Table 7. *Power to detect shifts in hormone peak time*

	Time Shift		
Hormone	1 h	2 h	3 h
Melatonin	0.98	0.99	0.99
Cortisol	0.74	0.99	0.99
PRA	0.20	0.62	0.92
Aldosterone	0.13	0.37	0.68

Bed rest studies have been used to simulate the lack of gravity in space (6). Increases in the amplitude of the peaks of PRA occurred at night after 80 h in bed, similar to conditions of zero gravity, likely due to loss of sodium and water and a decline in volume. PRA also was shown to increase with sleep without posture changes (2, 5). This alteration was not replicated in the present study, in which the PRA peak level, amplitude, mesor, and area were statistically unchanged after prolonged bed rest, notwithstanding the single subject who exhibited extremely high levels at baseline. However, the present intervention was 264 h compared with the previous intervention of 80 h, and it is possible that transient perturbations in hormone patterns occurred during short-term bed rest and that the patterns returned to baseline level by the end of the longer bed rest intervention.

In a study that frequently sampled plasma aldosterone, cortisol, and PRA in normal individuals on normal and low-sodium diets, it was concluded that these hormone secretions are synchronous, occur during late sleep and soon after arising, and may depend on ACTH secretion when the postural stimulus to renin is absent (16). The correlation of PRA with aldosterone was somewhat weaker. When ACTH and cortisol were suppressed with dexamethasone in one study (17), the aldosterone peaks did not change, suggesting that an external factor controls both ACTH and aldosterone and that renin is not the strongest determinant of aldosterone's rhythm. The present study confirmed the general time of the peak cortisol and aldosterone levels but also presented evidence that the peak level of aldosterone precedes the cortisol peak level by ~1 h. Given the fact that the plasma half-life of aldosterone is about one-third that of cortisol and that both are stimulated by

Table 9. *Circadian amplitude and waveform estimates*

	BL		PB		Δ			Correlation	
	Mean	SD	Mean	SD	Mean	SD	*P*	*r*	*P*
Melatonin, pg/ml									
Mesor	20.8	7.6	20.6	7.6	−0.1	4.8	0.97	0.81	0.008
Area	498	181	494	182	−1.7	115	0.97	0.81	0.008
Peak	65.9	23.7	67.0	27.5	2.6	13.8	0.59	0.87	0.003
Nadir	0.0	1.6	0.0	1.0	0.9	1.8	0.17	0.26	0.50
Rise	45.1	16.6	46.4	20.2	2.6	9.6	0.44	0.88	0.002
Fall	23.2	8.8	22.2	8.0	−1.0	6.2	0.65	0.75	0.02
Amplitude	68.4	24.5	68.6	27.7	1.7	14.8	0.75	0.85	0.003
PRA, $ng \cdot ml^{-1} \cdot h^{-1}$									
Mesor	2.1	1.8	2.0	0.5	−0.0	1.8	0.97	0.16	0.70
Area	49.4	42.6	48.9	11.1	−0.5	42.2	0.97	0.16	0.70
Peak	3.8	3.6	2.9	0.6	−0.9	3.5	0.48	0.25	0.55
Nadir	0.7	0.5	0.8	0.4	0.1	0.7	0.60	−0.23	0.58
Rise	1.7	1.9	0.8	0.2	−0.9	1.8	0.19	0.55	0.16
Fall	1.4	1.4	1.2	0.3	−0.2	1.1	0.69	0.81	0.02
Amplitude	3.1	3.2	2.0	0.5	−1.1	2.9	0.33	0.78	0.02
Aldosterone, ng/ml									
Mesor	5.6	1.8	5.9	1.8	0.5	0.8	0.11	0.89	0.001
Area	134	44	141	42	12	20	0.11	0.89	0.001
Peak	9.0	3.7	9.2	2.3	0.4	2.1	0.59	0.84	0.005
Nadir	3.0	1.0	3.5	1.1	0.6	0.9	0.08	0.65	0.06
Rise	3.4	2.0	3.3	1.0	−0.1	1.9	0.85	0.34	0.37
Fall	2.6	1.5	2.4	1.0	−0.1	0.2	0.78	0.63	0.07
Amplitude	6.0	3.3	5.6	1.8	−0.2	2.6	0.79	0.62	0.08
Cortisol, μg/dl									
Mesor	8.1	1.2	8.3	0.9	0.2	0.6	0.42	0.88	0.002
Area	194	28	198	22	3.8	14	0.42	0.88	0.002
Peak	15.9	2.6	15.7	2.0	−0.4	2.0	0.60	0.66	0.06
Nadir	2.1	1.1	1.6	1.5	−0.3	0.8	0.28	0.81	0.008
Rise	7.8	1.9	7.4	1.3	−0.5	1.6	0.35	0.57	0.30
Fall	5.9	0.9	6.7	1.4	0.5	1.1	0.24	0.47	0.20
Amplitude	13.7	2.2	14.1	2.1	−0.1	2.4	0.94	0.31	0.42

n, No. of subjects; Mesor, midline estimating statistic of rhythm; rise, peak − mesor; fall, Mesor − nadir; amplitude, rise + fall. Melatonin: $n = 10$, 9, and 9 for BL, PB, and Δ, respectively. PRA: $n = 8$, 8, and 8, for BL, PB, and Δ, respectively. Aldosterone: $n = 9$, 10, and 9 for BL, PB, and Δ, respectively. Cortisol: $n = 9$, 10, and 9 for BL, PB, and Δ, respectively. Area is expressed in unit of hormone times hour for 24 h. Analyses of mesor and area are equivalent.

ACTH, these data also support the possibility of a causal relationship. However, angiotensin II and its surrogate PRA could also be the mediator of the diurnal variation in aldosterone. Our data neither support nor refute this possibility.

In a head-down tilt bed rest study, the aldosterone pattern of subjects while ambulatory before and after 17 days of bed rest had a nocturnal depression, whereas during bed rest the nadir was earlier in the evening and there was a morning peak (19). The conclusion was that the aldosterone rhythm was abolished by head-down-tilt bed rest. Nevertheless, PRA and aldosterone levels are generally thought to increase during prolonged bed rest due to sodium loss (11). The aldosterone results in the present study extend that finding by showing that prolonged bed rest per se does not change the timing of the peaks significantly if posture is removed as a confounding variable. The pattern after 11 days of bed rest was similar to the pattern on the first day and matched the pattern observed previously (19), where there was a morning peak.

Studies seeking to characterize a circadian rhythm for PRA have reported conflicting results. Some have observed diurnal variation (6, 12). The finding of diurnal variation was extended by others who concluded that PRA may not be related to an intrinsic circadian process but rather that the oscillations are linked to the stages of sleep and are affected by meals (3). In the present study, we did not see consistent elevations in PRA at mealtimes. Other researchers observed rhythmicity before

Table 10. *Correlations among hormone peak levels and among the changes in peak levels due to prolonged bed rest*

	BL			PB			Δ		
Hormones	*n*	*r*	*P*	*n*	*r*	*P*	*n*	*r*	*P*
Melatonin with cortisol	9	−0.54	0.13	9	−0.46	0.22	8	0.72	0.04
Melatonin with PRA	8	−0.13	0.76	7	−0.09	0.84	7	−0.32	0.48
Melatonin with aldosterone	9	−0.22	0.56	9	−0.29	0.45	8	0.33	0.43
Cortisol with PRA	7	−0.52	0.22	8	0.56	0.15	7	−0.11	0.82
Cortisol with aldosterone	8	0.23	0.59	10	0.10	0.78	8	0.22	0.59
PRA with aldosterone	7	0.59	0.16	8	−0.29	0.48	7	0.51	0.24

n, No. of subjects.

and after but not during a 28-day bed rest period but did not observe a shift in timing (10). The present results replicated earlier conclusions that PRA peaks in the early morning (17, 20).

In previous studies, assertions of potential causality have been based in large part on observed correlations between pulses or secretions within and among individuals. By analyzing the temporal sequence of secretions, the present study can inform such assertions of causality. We first observed the sequence of the group mean peak secretions to be melatonin, then cortisol, then PRA, and then aldosterone. Formal statistical tests then enabled us to conclude that the sequence is most likely melatonin, then aldosterone, and then cortisol, with PRA being after melatonin and before cortisol but with an uncertain temporal relationship to aldosterone. Whereas more frequent sampling may help elucidate the direction of causality, these data suggest that it is unlikely that 11 days of bed rest desynchronizes the secretory sequentiality as a whole.

Multiple harmonic regression was a powerful tool for estimating features of the asymmetric hormone-secretion waveform. This method was most useful for estimating the time of the peak level, which is the relevant feature for the analysis of hormonal circadian rhythms. It may be less useful for estimating the time of the nadir if there are periods with levels close to zero with no change, as is the case with melatonin, and if pulsatility characteristics are more important than peak secretion timing. Nevertheless, the R^2 goodness-of-fit values for all subjects and conditions indicated that the model fits were excellent for all hormones and nearly perfect for melatonin.

Another advantage of the present study design besides the use of multiple harmonic regression to characterize the timing patterns was the removal of two types of confounding seen in earlier studies. In terms of diet, it was tightly controlled, with constant sodium, potassium, calcium, calories, and fluid content. In terms of posture, previous studies conducted baseline measurements while subjects were sitting, standing, or ambulatory, whereas we removed posture as a confounding variable by conducting both the baseline and prolonged bed rest measurements while the subjects were supine.

Prolonged bed rest, even with a 4° head-down tilt and in the presence of a volume shift, does not alter the diurnal rhythms of melatonin or the components of the RAAS in normal subjects. Under these conditions, cortisol rhythms are also not altered, suggesting that prolonged bed rest per se is not a significant enough stress factor to modify cortisol rhythm. Furthermore, because of the sensitivity and precision of melatonin as an indicator of the endogenous core rhythm, we hypothesized that prolonged bed rest may not modify the activity of the central nervous system circadian pacemaker. The results of this study have relevance in two areas. First, they suggest the abnormalities in the diurnal rhythms of the components of the RAAS seen in some clinical conditions are likely induced either by the stress of the clinical condition or, more likely, by a disruption in the normal activity of the circadian pacemaker. Given previous studies, this disruption is likely secondary to sleep disruption in the clinical setting. Second, for space travel, these results suggest that microgravity will not modify the hormone rhythms or even possibly the activity of the circadian pacemaker. Therefore, any alterations in these systems that are observed in space probably are secondary to the altered light-dark cycle or to the sleep disruption that commonly occurs.

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REFERENCES

1. **Ariznavarreta C, Cardinali DP, Villanua MA, Granados B, Martin M, Chiesa JJ, Golombek DA, and Tresguerres JA.** Circadian rhythms in airline pilots submitted to long-haul transmeridian flights. *Aviat Space Environ Med* 73: 445–455, 2002.
2. **Brandenberger G, Follenius M, Goichot B, Saini J, Spiegel K, Ehrhart J, and Simon C.** Twenty-four-hour profiles of plasma renin activity in relation to the sleep-wake cycle. *J Hypertens* 12: 277–283, 1994.
3. **Brandenberger G, Follenius M, Muzet A, Ehrhart J, and Schieber JP.** Ultradian oscillations in plasma renin activity: their relationships to meals and sleep stages. *J Clin Endocrinol Metab* 61: 280–284, 1985.
4. **Brown EN and Czeisler CA.** The statistical analysis of circadian phase and amplitude in constant-routine core-temperature data. *J Biol Rhythms* 7: 177–202, 1992.
5. **Charloux A, Gronfier C, Lonsdorfer-Wolf E, Piquard F, and Brandenberger G.** Aldosterone release during the sleep-wake cycle in humans. *Am J Physiol Endocrinol Metab* 276: E43–E49, 1999.
6. **Chavarri M, Ganguly A, Luetscher JA, and Zager PG.** Effect of bedrest on circadian rhythms of plasma renin, aldosterone, and cortisol. *Aviat Space Environ Med* 48: 633–636, 1977.
7. **Czeisler C and Khalsa SBS.** The human circadian timing system and sleep-wake regulation. In: *Principles and Practice of Sleep Medicine* (3rd ed.), edited by Kryger MH, Rogh T, and Dement WC. Philadelphia, PA: Saunders, 2000.
8. **Emanuel RL, Cain JP, and Williams GH.** Double antibody radioimmunoassay of renin activity and angiotensin II in human peripheral plasma. *J Lab Clin Med* 81: 632–640, 1973.
9. **Fraser S, Cowen P, Franklin M, Franey C, and Arendt J.** Direct radioimmunoassay for melatonin in plasma. *Clin Chem* 29: 396–397, 1983.
10. **Gauquelin G, Maillet A, Allevard AM, Sigaudo D, and Gharib C.** Diurnal rhythms of plasma renin activity, atrial natriuretic peptide and arterial pressure during head-down bed rest in humans. *Eur J Appl Physiol* 73: 536–543, 1996.
11. **Gharib C, Gauquelin G, Geelen G, Cantin M, Gutkovska J, Mauroux JL, and Guell A.** Volume regulating hormones (renin, aldosterone, vasopressin and natriuretic factor) during simulated weightlessness. *Physiologist* 28: S30–S33, 1985.
12. **Gordon RD, Wolfe LK, Island DP, and Liddle GW.** A diurnal rhythm in plasma renin activity in man. *J Clin Invest* 45: 1587–1592, 1966.
13. **Gronfier C, Chapotot F, Weibel L, Jouny C, Piquard F, and Brandenberger G.** Pulsatile cortisol secretion and EEG delta waves are controlled by two independent but synchronized generators. *Am J Physiol Endocrinol Metab* 275: E94–E100, 1998.
14. **Gronfier C, Simon C, Piquard F, Ehrhart J, and Brandenberger G.** Neuroendocrine processes underlying ultradian sleep regulation in man. *J Clin Endocrinol Metab* 84: 2686–2690, 1999.
15. **Jones B, Jarvis P, Lewis JA, and Ebbutt AF.** Trials to assess equivalence: the importance of rigorous methods. *BMJ* 313: 36–39, 1996.
16. **Katz FH, Romfh P, and Smith JA.** Episodic secretion of aldosterone in supine man; relationship to cortisol. *J Clin Endocrinol Metab* 35: 178–181, 1972.
17. **Katz FH, Romfh P, and Smith JA.** Diurnal variation of plasma aldosterone, cortisol and renin activity in supine man. *J Clin Endocrinol Metab* 40: 125–134, 1975.
18. **Melada GA, Goldman RH, Luetscher JA, and Zager PG.** Hemodynamics, renal function, plasma renin, and aldosterone in man after 5 to 14 days of bedrest. *Aviat Space Environ Med* 46: 1049–1055, 1975.

19. **Millet C, Custaud MA, Allevard AM, Zaouali-Ajina M, Monk TH, Arnaud SB, Claustrat B, Gharib C, and Gauquelin-Koch G.** Influence of head-down bed rest on the circadian rhythms of hormones and electrolytes involved in hydroelectrolytic regulation. *Eur J Appl Physiol* 85: 74–81, 2001.
20. **Modlinger RS, Sharif-Zadeh K, Ertel NH, and Gutkin M.** The circadian rhythm of renin. *J Clin Endocrinol Metab* 43: 1276–1282, 1976.
21. **Modlinger RS, Sharif-Zadeh K, Schneider G, and Gutkin M.** Circadian rhythm of plasma renin activity in primary hyperaldosteronism. *J Clin Endocrinol Metab* 42: 361–364, 1976.
22. **Opstad K.** Circadian rhythm of hormones is extinguished during prolonged physical stress, sleep and energy deficiency in young men. *Eur J Endocrinol* 131: 56–66, 1994.
23. **Richards AM, Nicholls MG, Espiner EA, Ikram H, Cullens M, and Hinton D.** Diurnal patterns of blood pressure, heart rate and vasoactive hormones in normal man. *Clin Exp Hypertens A* 8: 153–166, 1986.
24. **Samel A, Wegmann HM, and Vejvoda M.** Response of the circadian system to 6 degrees head-down tilt bed rest. *Aviat Space Environ Med* 64: 50–54, 1993.
25. **Selye H.** *The Physiology and Pathology of Exposure to Stress*. Montreal, Canada: Asta, 1950.
26. **Shanahan TL and Czeisler CA.** Light exposure induces equivalent phase shifts of the endogenous circadian rhythms of circulating plasma melatonin and core body temperature in men. *J Clin Endocrinol Metab* 73: 227–235, 1991.
27. **van Loon JH.** Diurnal body temperature curves in shift workers. *Ergonomics* 6: 267–273, 1963.
28. **Vernikos-Danellis J, Leach CS, Winget CM, Rambaut PC, and Mack PB.** Thyroid and adrenal cortical rhythmicity during bed rest. *J Appl Physiol* 33: 644–648, 1972.
29. **Weitzman ED, Zimmerman JC, Czeisler CA, and Ronda J.** Cortisol secretion is inhibited during sleep in normal man. *J Clin Endocrinol Metab* 56: 352–358, 1983.
30. **Williams GH, Cain JP, Dluhy RG, and Underwood RH.** Studies of the control of plasma aldosterone concentration in normal man. I. Response to posture, acute and chronic volume depletion, and sodium loading. *J Clin Invest* 51: 1731–1742, 1972.
31. **Winget CM, Vernikos-Danellis J, Cronin SE, Leach CS, Rambaut PC, and Mack PB.** Circadian rhythm asynchrony in man during hypokinesis. *J Appl Physiol* 33: 640–643, 1972.