Existence of an Endogenous Circadian Blood Pressure Rhythm in Humans That Peaks in the Evening

Short Communication

Steven A. Shea, Michael F. Hilton, Kun Hu, Frank A.J.L. Scheer

Rationale: Blood pressure (BP) usually decreases during nocturnal sleep and increases during daytime activities. Whether the endogenous circadian control system contributes to this daily BP variation has not been determined under appropriately controlled conditions.

Objective: To determine whether there exists an endogenous circadian rhythm of BP in humans.

Methods and Results: In 28 normotensive adults (16 men), we assessed BP across 3 complementary, multiday, in-laboratory protocols performed in dim light, throughout which behavioral and environmental influences were controlled and/or uniformly distributed across the circadian cycle via: (1) a 38-hour “constant routine,” including continuous wakefulness; (2) a 196-hour “forced desynchrony” with 7 recurring 28-hour sleep/wake cycles; and (3) a 240-hour forced desynchrony with 12 recurring 20-hour sleep/wake cycles. Circadian phases were derived from core body temperature. Each protocol revealed significant circadian rhythms in systolic and diastolic BP, with almost identical rhythm profiles among protocols. The peak-to-trough amplitudes were 3 to 6 mm Hg for systolic BP and 2 to 3 mm Hg for diastolic BP (always P<0.05). All 6 peaks (systolic and diastolic BP in 3 protocols) occurred at a circadian phase corresponding to ∼9:00 PM (ie, the biological evening). Based on substantial phase differences among circadian rhythms of BP and other variables, the rhythm in BP appeared to be unrelated to circadian rhythms in cortisol, catecholamines, cardiac vagal modulation, heart rate, or urine flow.

Conclusions: There exists a robust endogenous circadian rhythm in BP. The highest BP occurred at the circadian time corresponding to ∼9:00 PM, suggesting that the endogenous BP rhythm is unlikely to underlie the well-documented morning peak in adverse cardiovascular events. (Circ Res. 2011;108:980-984.)

Key Words: circadian ■ blood pressure, humans ■ myocardial infarction ■ stroke

Numerous epidemiological studies reveal a profound morning increase in the incidence of adverse cardiovascular events, including sudden cardiac death, ventricular arrhythmia, stroke,1 and myocardial infarction.2 The extent to which these peaks are caused by a day/night pattern of behaviors and/or endogenous circadian factors is unknown.3 The circadian timing system orchestrates endogenous circadian rhythms in physiology and behavior and is composed of the master circadian pacemaker located in the suprachiasmatic nucleus and circadian oscillators in peripheral tissues.4 The suprachiasmatic nucleus may influence the cardiovascular system via multisynaptic neural projections to the heart, adrenal cortex, adrenal medulla, kidneys, and vasculature and resultant neural or endocrine effects,5 and secondarily by circadian influences on behaviors, such as activity levels, alertness, and sleep. Moreover, recent animal investigations have also documented the actions of molecular circadian clocks in peripheral tissues that can affect blood pressure (BP),6 ischemia/reperfusion tolerance,7 and vascular remodeling.8 In contrast, mechanistic circadian studies of cardiovascular function in humans are sparse.

A primary risk factor for adverse cardiovascular events is elevated arterial BP.9 Countless studies have documented the day/night variation of BP in humans, which has been used to classify hypertensive patients into nocturnal “dippers” (≥10% drop in BP overnight) and “nondippers,” and it has been shown that nondippers are at increased risk for serious adverse events.10 However, no studies have adequately studied the relative contributions to this day/night BP variation from the endogenous circadian cycle and from the daily changes in behaviors, such as the sleep/wake cycle. Thus, we tested the hypothesis that there exists an endogenous circadian rhythm in BP in humans.

Methods

An expanded Methods section is available in the Online Data Supplement at http://circres.ahajournals.org.
subjects maintained a regular sleep/wake schedule for 2 to 3 weeks before entering the laboratory, followed by 2 baseline days and nights in the laboratory (16-hour scheduled wakefulness, 8-hour scheduled sleep, both at home and in the laboratory). Thereafter, to avoid resetting the phase of the circadian system, all laboratory protocols were performed in dim light (<4 lux).11 Subjects completed 1 or 2 of the 3 protocols: (1) 38-hour “constant routine protocol” (CR), with continuous wakefulness, semirecumbency, and 2-hourly isocaloric snacks (Figure 1, top); (2) 196-hour forced desynchrony with 7 recurring 28-hour sleep/wake cycles (FD28) (Figure 1, middle: 18-hour 40-minute wakefulness, 9-hour 20-minute sleep); or (3) 240-hour forced desynchrony with 12 recurring 20-hour sleep/wake cycles (FD20) (Figure 1, bottom: 13-hour 20-minute wakefulness, 6-hour 40-minute sleep). In essence, the CR abolished sleep and minimized behaviors, whereas the FD28 and FD20 maintained a normal sleep: awake ratio of 1:2 and scheduled all activities so that they became uniformly distributed across the circadian cycle.

Measurements and Analyses

Subjects wore a flexible rectal temperature sensor for measurement of CBT, which was used as the circadian phase marker.12 For each subject, the fitted CBT minimum was assigned as a reference phase marker of 0°. BP was measured repeatedly by automatic oscillometric cuff sphygmomanometer from an upper arm. Heart rate was also measured in each protocol. These measurements were made throughout periods of relaxed wakefulness during each protocol. Data were assigned a circadian phase and the existence of any circadian rhythms were tested by cosinor mixed-model ANOVA. To assess whether the rhythms were robust, the phases of peaks and the amplitudes of the circadian rhythms of BP and heart rate were compared across protocols using unpaired t-tests. To gain insight into control mechanisms across the circadian cycle, most of the following potentially related variables were measured throughout each protocol: cardiac vagal modulation (estimated from high frequency power of interbeat interval variability), plasma cortisol, urinary or plasma catecholamines, and urine flow. The phases of peaks and troughs of these potentially related variables were compared with the circadian rhythm of BP using paired t-tests. Further details are provided in the Online Methods, Online Table I, Online Figure I.

Results

Each protocol revealed significant circadian rhythms in systolic and diastolic BP, with almost identical rhythm amplitudes and phase relationships among protocols (Figures 2 and 3; Online Figure I and Online Table I). For the 3 protocols, the peak-to-trough amplitudes were 3 to 6 mm Hg for systolic BP and 2 to 3 mm Hg for diastolic BP (always P<0.05). All 6 peaks (systolic and diastolic BP in 3 protocols) occurred in the circadian phase range of 219° to 265°, with an average phase of 244°, equivalent to ~9:00 PM (Online Table I). The average circadian phase of the peak in heart rate across the 3 protocols was 161°, which is ~6 hours earlier than the

Figure 1. Three complementary protocols used to examine underlying circadian rhythmicity of BP. Three protocols designed to keep behaviors constant across the circadian cycle (top graph, 2 baseline days followed by 38-hour constant routine while semirecumbent and awake) or to evenly distribute behaviors across all circadian phases (middle graph, 7 recurring 28-hour behavioral cycles [28-hour forced desynchrony]; bottom graph, 12 recurring 20-hour behavioral cycles [20-hour forced desynchrony]). In each graph, subsequent days are “double-plotted” to the right and below prior days to visually aid protocol continuity. The x axes: clock times for an example subject having an habitual wake time of 8:00 AM. Black boxes indicate scheduled sleep episodes in darkness; gray/hatched bars, scheduled wakefulness in dim light conditions (<4 lux) to avoid circadian rhythm resetting.11

Subjects gave informed consent, and the studies were approved by the local Human Research Committee. We studied 28 adults (16 men) who were normotensive, nonobese, and healthy (other than mild asthma, n=6), and who took no medications (other than oral contraceptives and β2-agonist rescue inhalers for asthma), and no caffeine, alcohol, or nicotine products for 2 to 3 weeks immediately before and throughout the laboratory studies. The subjects with asthma participated in only 1 of 3 protocols and all data in the 4 hours following any rescue inhaler use were excluded from analyses (see the Online Data Supplement).

Protocols

Quantifying the effect of the endogenous circadian system on BP requires controlling all environmental factors and behaviors that can affect either BP or the circadian cycle (eg, activity, posture, meals, sleep, room temperature, light) while measuring BP across the circadian cycle and measuring an endogenous circadian phase marker whose effects can be mathematically isolated from any influences caused by daily behavioral changes (eg, core body temperature [CBT], rather than activity levels, which have been used in most animal studies and greatly affect BP). This was achieved by measuring BP throughout 3 separate and complementary circadian protocols, as shown in Figure 1. To stabilize circadian rhythms,
average circadian peak in BP. During the CR, there were substantial and significant differences between the timing of the circadian peak in BP (systolic and diastolic) and all other potentially relevant circadian rhythms measured, including circadian peaks in heart rate, urinary catecholamines and plasma cortisol, and circadian troughs in urine flow and cardiac vagal modulation (Figure 3 and Online Table I). Similarly, results for these variables during the 2 forced desynchrony protocols (where available) were highly consistent with the CR data (see Online Table I and Online Figure I). Thus, the endogenous circadian rhythm in BP appeared to be unrelated to circadian rhythms in cortisol, catecholamines, cardiac vagal modulation, heart rate, or urine flow.

**Discussion**

The present study has revealed an endogenous circadian rhythm of BP. This study yielded robust results that were almost identical in 3 groups of subjects during 3 different protocols. This study was performed because of the possible relevance of endogenous circadian BP rhythms to the day/night pattern of adverse cardiovascular events. Our results are perhaps unexpected because the timing of the endogenous circadian peak in BP occurs in the evening, whereas the lowest circadian BP occurs around the most vulnerable time for adverse cardiovascular events. Thus, our data suggest that the morning peaks in adverse cardiovascular events are not caused by circadian rhythm-related increases in BP. Presumably, endogenous circadian rhythms in other cardiovascular variables (eg, platelet function, sympathovagal balance, and endothelial function) and/or physiological responses to day/night patterns of behavioral changes are more important in this regard. We note that wakening in the evening, as can occur with shift work, jet lag, and sleep disorders, may result in an exaggerated BP surge attributable to summed behavioral- and circadian-related increases in BP, perhaps helping to explain the curious secondary evening peak in incidence of myocardial infarction in vulnerable individuals. Moreover, there may be more than simple summation of these effects, whereby the circadian system modulates the BP response to a standardized stress.

This study was performed in healthy subjects, and it remains to be seen whether the endogenous circadian morning trough in BP that we observed could increase the risk of ischemic stroke at that time in people with existing carotid or cerebral artery stenoses and whether the amplitudes or phases of the endogenous circadian BP rhythms are abnormal in more vulnerable groups.

There are many studies that refer to a “circadian rhythm” of BP in humans and other animals without clarifying whether this is an endogenous rhythm, and/or physiological responses to day/night patterns of behavioral changes are more important in this regard. We note that wakening in the evening, as can occur with shift work, jet lag, and sleep disorders, may result in an exaggerated BP surge attributable to summed behavioral- and circadian-related increases in BP, perhaps helping to explain the curious secondary evening peak in incidence of myocardial infarction in vulnerable individuals. Moreover, there may be more than simple summation of these effects, whereby the circadian system modulates the BP response to a standardized stress.

This study was performed in healthy subjects, and it remains to be seen whether the endogenous circadian morning trough in BP that we observed could increase the risk of ischemic stroke at that time in people with existing carotid or cerebral artery stenoses and whether the amplitudes or phases of the endogenous circadian BP rhythms are abnormal in more vulnerable groups.

Figure 2. Similar endogenous circadian variations in BP across 2 complementary forced desynchrony protocols. Shown are (means ± SEM) systolic BP (SBP), diastolic BP (DBP), and heart rate (HR) expressed in absolute units (left axes) and as percentages of individual averages (right axes). Data are aligned according to circadian phase (x axis) and plotted in 60° bins (equivalent to ~4 hours). Corresponding approximate clock time is shown on the top x axis. 0° represents CBT minimum (~5:00 AM in these subjects). Thin gray shaded bars are shown at the top of each graph to indicate the average equivalent clock time when subjects would normally sleep when at home (although all data were collected during wakefulness in the laboratory). Solid lines represent the cosinor model fit and the probability values indicate significance of circadian rhythmicity. The phases of the peak SBP are shown by dashed vertical lines in each protocol and occurred close to 240° (equivalent to ~9:00 PM), which was similar to the timing of the peaks in DBP, but quite distinct from the timing of the circadian peaks in HR.
ought to help clarify some misperceptions in the field because of such methodological and interpretative problems (see discussion in the Online Data Supplement).

Regarding mechanisms, there were notable circadian rhythms in numerous variables that would be expected to correlate in the short term with acute changes in BP, including cortisol, catecholamines, cardiac vagal modulation, heart rate, and urine flow (likely inversely related to blood volume). However, based on the substantial phase differences among these rhythms, the endogenous circadian evening peaks in systolic and diastolic BP did not appear to be mechanistically linked with the endogenous \( \sim 24 \) rhythmicity of these other variables. This is an intriguing result and points to different control mechanisms that have yet to be elucidated and that must regulate BP across the circadian cycle.

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**Disclosures**
None.

**References**


Novelty and Significance

What Is Known?
- Numerous epidemiological studies have shown a profound morning increase in the incidence of adverse cardiovascular events, including sudden cardiac death, ventricular arrhythmia, stroke, and myocardial infarction.
- A primary risk factor for adverse cardiovascular events is elevated arterial blood pressure (BP), which usually decreases during nocturnal sleep and increases during daytime activities.
- The endogenous circadian timing system orchestrates daily rhythms in physiology (regardless of ongoing behaviors such as sleep and wake cycle) and could potentially contribute to the day/night pattern of changing BP.

What New Information Does This Article Contribute?
- The data show presence of a robust endogenous circadian rhythm in BP.
- The highest BP occurred at the circadian time corresponding to ~9 PM, suggesting that the endogenous BP rhythm is unlikely to underlie the well-documented morning peak in adverse cardiovascular events.
- The endogenous circadian evening peaks in systolic and diastolic BP did not appear to be mechanistically linked with the endogenous ~24 rhythmicity of numerous variables that would be expected to correlate in the short term with acute changes in BP, including cortisol, catecholamines, cardiac vagal modulation, and heart rate.

Arterial BP usually decreases during sleep and increases during daytime activities. Many epidemiological studies have shown a large morning increase in adverse cardiovascular events, potentially related to elevated arterial BP around that time. We discovered the presence of an endogenous circadian rhythm in BP that can contribute to the day/night BP variation. However, the endogenous circadian peak in BP occurred at ~9 PM, suggesting that the circadian BP rhythm is unlikely to underlie the morning peak in adverse cardiovascular events. Presumably, circadian rhythms in other cardiovascular variables (eg, platelet or endothelial function) and/or physiological responses to day/night patterns of behaviors (such as awakening) may be more related to the day/night pattern of adverse events. The endogenous circadian rhythm in BP was not associated with circadian rhythmicity of numerous variables that normally correlate with acute changes in BP, including cortisol, catecholamines, cardiac vagal modulation, and heart rate. This suggests that a different control mechanism that has yet to be elucidated regulates BP across the circadian cycle.
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SUPPLEMENTAL MATERIAL

Existence of an Endogenous Circadian Blood Pressure Rhythm in Humans that Peaks in the Evening

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Detailed Methods:

Quantifying the independent effect of the endogenous circadian system on blood pressure (BP) requires controlling environmental factors and behaviors that can affect BP or the circadian cycle (e.g., activity, posture, meals, sleep, room temperature, light) while measuring BP across the entire circadian cycle. A reliable circadian phase marker that is relatively independent of behavior is required. Such a marker is the circadian component of core body temperature (CBT). Circadian rhythms can be extracted from CBT measurements when either minimizing any masking influences of behaviors, as is achieved in the constant routine protocol, or distributing the masking influences uniformly across the circadian cycle, as is achieved in the forced desynchrony protocols (rather than using activity levels as a circadian phase marker which has been used in most animal studies). This was achieved by analysis of BP data collected in three separate and complementary valid circadian protocols that had different primary aims, while all three protocols had the common secondary aim of determining the existence and extent of any endogenous circadian rhythm of BP, as presented herein.

Subjects

All subjects gave written informed consent and the studies were approved by the local Human Research Committee. There were 28 subjects (16 men, 12 women; mean age 26±1 year [range 19-44 year]; BMI <30 kg/m². Prior to enrollment in the studies, participants underwent extensive screening to ensure they had no current physical or mental disorders (other than mild asthma, n = 6). The screening test battery included medical history, physical and psychological examination, psychological questionnaires, electrocardiogram (ECG), and biochemical analysis of blood and urine. To be eligible for participation in the protocols subjects had to be non-obese (BMI <30) and taking no medications (other than rescue inhaler medication in subjects with asthma, and oral contraceptive pills in females). The two groups of subjects for the 20-h forced desynchrony and the constant routine protocols were homogeneous, namely very healthy young, non-obese controls, without any medical disorders or medications. Subjects with asthma were included in only one of the three protocols - the 28-h forced desynchrony protocol (see below). Only subjects with asthma who used an inhaled β2-agonist as their sole medication were recruited. Additional exclusion criteria for subjects with asthma were use of inhaled or topical steroids in the past 8 weeks, use of oral steroids in the past year, or acute asthma exacerbation in the past 6-weeks. Overall, the 6 subjects with mild asthma used 0.2 mg Albuterol β2-agonist rescue inhaler only 13 times over 784 h of wakefulness recording (approximately once per 60 h of wakefulness). Published data indicate that such medication use does not affect BP in healthy subjects, and any effects on heart rate subside within 90 min. Thus, to ensure that there were no effects of inhaler use on BP or heart rate in our data, we excluded any data from the 4 h following inhaler from all analyses. A limitation of the 28-h forced desynchrony protocol was that there were insufficient subjects in each group for a meaningful comparison between those subjects with asthma (n = 6) and without asthma (n = 5).
Study Protocols

Ambulatory Protocol: To ensure a stable circadian phase with respect to the time of day at baseline, shift work within three years and crossing more than one time zone within three months of the study was exclusionary. In addition, all subjects maintained a regular sleep/wake schedule with 8-h sleep starting at the same time each night for 2-3 weeks immediately prior to admission to the laboratory, as verified by sleep/wake diaries, call-in times to a time-stamped voice recorder and wrist actigraphy (Activwatch; Minimitter, Bend, OR). Urine toxicology screens upon admission confirmed that subjects were free of any drugs (except inhaler medication in subjects with asthma), including caffeine, alcohol and nicotine.

Laboratory Protocols: The three complementary protocols are shown in Figure 1 of the main manuscript. To avoid resetting the phase of the circadian system, all protocols were performed in dim light (0 lux during scheduled sleep and <4 lux during wakefulness). Following two baseline days and nights (16-h of scheduled wakefulness and 8-h of scheduled sleep), subjects completed one of the three protocols: (i) a 38-h ‘constant routine’ including continuous wakefulness, semi-recumbency, and 2-hourly isocaloric snacks (including identical amounts of ingested fluid each 2-h) (CR; n = 9 subjects); (ii) a 196-h ‘forced desynchrony’ with seven recurring 28-h sleep/wake cycles with 18h 40-min of scheduled wakefulness and 9h 20-min of scheduled sleep (FD28 protocol; n = 11 subjects); or (iii) a 240-h ‘forced desynchrony’ with twelve recurring 20-h sleep/wake cycles with 13h 20-min of scheduled wakefulness and 6h 40-min of scheduled sleep (FD20 protocol, n = 12 subjects). Overall, there were 32 studies performed in the 28 subjects, with 4 subjects performing both the CR and the FD28 studies. In essence, the CR protocol abolished sleep and minimized behavioral changes, whereas the FD20 and FD28 protocols maintained a normal wake:sleep ratio of 2:1, and scheduled all activities, sleep and wake episodes and physiological testing such that these became uniformly distributed across the circadian cycle by the end of the protocol. This uniform distribution of behaviors permits independent assessment of underlying circadian rhythmicity while controlling for any effects of behaviors on BP.

Measurements and Analyses

Subjects wore a flexible rectal temperature sensor (Yellow Springs Instrument Company, OH, USA) continuously throughout each protocol (except for during bowel movements and showers) for measurement of core body temperature (CBT), which was used as the circadian phase marker. Non-orthogonal Cosinor analyses of an individual’s CBT data were used to estimate each subject’s circadian period and circadian phase (except for the CR data where circadian period was assumed to be 24.18-h based on prior results from this laboratory and/or the circadian period revealed during the FD28 protocol in the 4 subjects who performed both FD28 and CR). The average circadian period in this group of subjects during the two forced desynchrony protocols was 24.17±0.04-h [23.8-24.6-h]. Fitted circadian CBT minimum was assigned as the reference phase marker of 0° for each individual. The average clock time of the circadian CBT minimum was 04.58h ± 50-min for the CR protocol, 04.56h ± 21-min for the FD28 protocol and 4.55h ± 19-min for the FD20 protocol, thus all CBT minima were very close to 5 AM. Using the information on the circadian period and the timing of the circadian phase marker (CBT minimum = 0°), all data were then assigned a specific circadian phase (0°-360°).

Blood pressure was measured repeatedly by automatic oscillometric cuff sphygmomanometer (Dinamap, Critikon INC, Tampa, FL) from a upper arm every 3-6-min throughout test batteries that were scheduled during the wake periods of each protocol. An electrocardiogram was also
recorded in each protocol for assessment of heart rate using semi-automatic R-R interval detection. Furthermore, the following variables were measured during the CR and the FD28 protocols; cardiac vagal modulation (estimated from high frequency power of the inter-beat interval variability), plasma cortisol, urinary catecholamines and urine flow (this was measured because it is known to have a circadian rhythm and could be inversely related to blood volume given that ingested fluid was taken at a constant rate throughout the CR protocol, and circulating blood volume could affect BP). The same variables were also measured during the FD20 protocol with the exceptions that plasma catecholamines were assayed instead of urinary catecholamines, and urine flow was not quantified. In the 38-h CR, a test battery was performed every 2h which consisted of computerized vigilance tests, pulmonary function tests and periods of rest for assessment of basal physiological function – including BP. Similarly, throughout the 196-h FD28, an identical test and measurement battery was performed every 4-h during scheduled wakefulness. In contrast, during the FD20 there was an entirely different battery of standardized cognitive and physical challenges comprising a computerized serial addition test (cognitive challenge: 20-min baseline, 10-min computerized test, 20-min recovery), a tilt table test (postural challenge; 25-min baseline while supine, 15-min 60-degrees passive head-up tilt, 20-min recovery while supine), and cycling on ergometer (exercise challenge; 25-min baseline, 15-min cycling at 60% maximum heart rate [calculated from 220 beats/min minus age in years], and 20-min recovery). In the FD20 this test battery was performed once per 20-h day starting 2h 55min after each scheduled awakening (as compared to resting test periods for BP measurements every 2 to 4h in the other two protocols). Only resting baseline data from each of these three tests during the FD20 were used for the current analyses. Changes in the cardiovascular responses to exercise and to tilt tests across the circadian cycle have been previously published. Subsets of the epinephrine and cortisol data from the FD28 have been previously published in a different format in Figure 2, page 4454 in Scheer et al 2009, and part of the heart rate data from the FD28 protocol has been previously published (as mean R-R interval rather than heart rate) in Panel G of Figure 2, page 20704 in Ivanov et al 2007).

In each protocol, data were averaged within each test window and assigned a circadian phase according to the middle of the test battery or challenge. Data from the CR and FD28 were assessed from data collected when subjects were semi-recumbent and at rest, whereas in the FD20, data were averaged across each baseline condition (i.e., prior to mental stress while semi-recumbent, prior to the tilt-table test while supine, and prior to the exercise test while seated at rest on a cycle ergometer).

The existence of circadian rhythms in all physiological variables were tested by using Cosinor analyses including a fundamental circadian component (~24-h), a harmonic component (~12-h), time into the study, and, for the FD protocols, the measurement windows within each wake episode as fixed factors based on mixed model analyses of variance (JMP, SAS Institute). Unpaired t-tests were used to statistically compare the amplitudes and phases of peaks and troughs of the circadian rhythms derived from Cosinor analyses between protocols (constant routine, 28-h forced desynchrony and 20-h forced desynchrony) for the same variables – to assess the robustness of circadian rhythmicity of each variable. Paired t-tests were used were used to statistically compare the phases of peaks and troughs of the circadian rhythms between variables within the same protocol – to assess the potential mechanistic relationship between variables. The results of these analyses are shown below in Online Supplemental Material Table I. Similarly, the Cosinor fits are portrayed below in Online Supplemental Material Figure I for visual comparisons between protocols within each variable, and between variables within the same protocol.
Comparison of results of current study with other published reports:

There are clear methodological and interpretative differences in some of the published literature regarding circadian rhythms and BP regulation. For instance, there are many studies that refer to a ‘circadian rhythm’ of BP in humans and other animals without clarifying whether this is an endogenous rhythm, but almost all such studies made BP measurements across the 24-h period while permitting sleep and altered activity across the day and night. Thus, the known reduction in BP caused by sleep and the increase in BP caused by activity are behavioral rather than endogenous explanations for much of the observed day/night pattern in BP, which is usually characterized by a peak in the middle of the wake/activity period and trough during sleep.\(^{10-12}\) Lesion of the SCN in rats does abolish the day/night pattern of BP but also abolishes the regular activity patterns,\(^{13}\) thus failing to demonstrate a circadian BP rhythm independent of activity (despite opposite claims in the literature\(^ {12}\)). In contrast, our demonstration of a clear endogenous circadian BP rhythm while controlling for activity\(^ {4}\) suggests that the previously observed day/night patterns of BP could be caused by the summation of endogenous circadian and behavioral effects on BP. In contrast to the current results, two studies have attempted to assess endogenous circadian rhythmicity of BP in humans by using a ‘constant routine’ protocol, but with negative results.\(^ {14-15}\) These two studies had several methodological limitations including: (1) absence of an endogenous circadian phase marker (clock time was used as a surrogate phase marker, which introduces errors when averaging data from subjects with different relationships between circadian phase and clock time, potentially obscuring an underlying circadian rhythmicity); (2) subjects were exposed to light at up to 100 lux during the ‘constant routines’, and this degree of light exposure has a major effect on circadian phase and amplitudes;\(^ {8}\) (3) such light exposure also suppresses melatonin production during the circadian night,\(^ {8}\) potentially cancelling any melatonin-mediated nocturnal dip in BP;\(^ {16}\) and (4) subjects were allowed to move around between measurements, which presumably introduced uncontrolled and variable effects on BP. Our study overcame these limitations and yielded robust results that were almost identical in three groups of subjects during three different protocols, revealing an endogenous evening peak in systolic and diastolic BP.

Supplemental References:


### Supplemental Material Table I:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Circadian rhythmicity (phases and amplitudes)</th>
<th>Constant Routine (CR)</th>
<th>28-h Forced Desynchrony (FD28)</th>
<th>20-h Forced Desynchrony (FD20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
</tr>
<tr>
<td>Systolic Blood Pressure</td>
<td>[Circadian Peak (º)]</td>
<td>251 (16)</td>
<td>255 (24)</td>
<td>229 (9)</td>
</tr>
<tr>
<td></td>
<td>[Peak-trough (mmHg)]</td>
<td>5.9 (2.0)</td>
<td>3.2 (1.4)</td>
<td>4.5 (0.8)</td>
</tr>
<tr>
<td>Diastolic Blood Pressure</td>
<td>[Circadian Peak (º)]</td>
<td>247 (18)</td>
<td>265 (14)</td>
<td>219 (10)</td>
</tr>
<tr>
<td></td>
<td>[Peak-trough (mmHg)]</td>
<td>3.2 (1.4)</td>
<td>2.4 (0.9)</td>
<td>2.3 (0.5)</td>
</tr>
<tr>
<td>Heart Rate</td>
<td>[Circadian Peak (º)]</td>
<td>133 (20) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>155 (32) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>194 (11) &lt;sup&gt;a,b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>[Peak-trough (beats/min)]</td>
<td>8.0 (1.0)</td>
<td>3.7 (0.8) &lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.9 (0.7) &lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma Cortisol</td>
<td>[Circadian Peak (º)]</td>
<td>57 (4) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>67 (3) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>59 (4) &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>[Peak-trough (µg/dl)]</td>
<td>13.3 (0.8)</td>
<td>9.8 (0.4)</td>
<td>7.3 (0.4)</td>
</tr>
<tr>
<td>Epinephrine (urinary excretion</td>
<td>[Circadian Peak (º)]</td>
<td>108 (9) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>192 (34) &lt;sup&gt;b&lt;/sup&gt;</td>
<td>107 (23) &lt;sup&gt;a,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>or plasma conc.)</td>
<td>[Peak-to-trough (µg/h; plasma: pg/ml)]</td>
<td>0.51 (0.05)</td>
<td>0.28 (0.03)</td>
<td>10.5 (2.8)</td>
</tr>
<tr>
<td>Norepinephrine (urinary excretion or plasma conc.)</td>
<td>[Circadian Peak (º)]</td>
<td>163 (5) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>NS</td>
<td>166 (46)</td>
</tr>
<tr>
<td></td>
<td>[Peak-to-trough (µg/h; plasma: pg/ml)]</td>
<td>0.31 (0.12)</td>
<td>NS</td>
<td>39.8 (10.1)</td>
</tr>
<tr>
<td>Urine flow</td>
<td>[Circadian Trough (º)]</td>
<td>296 (4) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>310 (23)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>[Peak-trough (ml/h)]</td>
<td>136 (9)</td>
<td>98 (21)</td>
<td>NA</td>
</tr>
<tr>
<td>Cardiac vagal modulation (lnHF)</td>
<td>[Circadian Trough (º)]</td>
<td>143 (15) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>NS</td>
<td>188 (96)</td>
</tr>
<tr>
<td></td>
<td>[Peak-trough (lns²)]</td>
<td>0.23 (0.06)</td>
<td>NS</td>
<td>0.12 (0.03)</td>
</tr>
</tbody>
</table>

**Legend**: Circadian phases and amplitudes of physiological variables across three protocols (constant routine [CR], 28-h forced desynchrony [FD28] and 20-h forced desynchrony [FD20]) derived from Cosinor analyses with 0° corresponding to the circadian phase marker of core body temperature minimum, and 360° representing one circadian period (~24 h). Reading across, there are very similar circadian rhythms of systolic blood pressure (circadian amplitudes and phases of circadian peaks), as well as most other variables, between the three protocols. Reading down, during the CR protocol there were substantial and significant differences between the timing of the circadian peak in BP (systolic and diastolic) and all other potentially relevant circadian rhythms measured, including circadian peaks in heart rate, urinary catecholamines and plasma cortisol, and circadian troughs in urine flow and cardiac vagal modulation. For instance, the circadian phase of the peak in heart rate is 133°, which is 118° (~8 h) earlier than the circadian peak in BP. Overall, results during the two forced desynchrony protocols were highly consistent with the CR data, although the significance levels showed variation between protocols such that these similarities are more evident when data are superimposed (see Supplemental Material Figure I below). The shortest phase difference between relevant peaks or troughs was the circadian phase of the peak in heart rate during FD20 (194°) which occurred 34° (~2.25 h) earlier than the circadian peak in BP, and this phase difference was still significant (p=0.02). Thus: (1) there were similarities in circadian rhythms of each variable across protocols (with the exceptions that there were no significant circadian rhythms detected in urinary norepinephrine or cardiac vagal modulation in the FD28 protocol), suggesting that these rhythms are robust; and (2) there are large phase lags among variables within protocols, indicating that the circadian peak in BP is not systematically related to underlying circadian peaks in cortisol, catecholamines, or heart rate, or to underlying circadian troughs in urine flow or cardiac vagal modulation.

**NS**: no significant circadian rhythm detected with Cosinor analysis; **NA**: data not available; **<sup>a</sup> p<0.05** phases of circadian peaks of systolic BP vs. peaks (or troughs) of other variables within the same protocol; **<sup>b</sup> p<0.05** CR vs. FD28 protocols; **<sup>c</sup> p<0.05** CR vs. FD20 protocols; **<sup>d</sup> p<0.05** FD28 vs. FD20 protocols.
Supplemental Material Figure I. Similar endogenous circadian physiological variations revealed in three complementary protocols. Circadian variations in systolic BP (SBP), diastolic BP (DBP), heart rate (HR), catecholamines, plasma cortisol, cardiac vagal modulation (lnHF from high frequency power of inter-beat intervals) and urine flow rate in 3 protocols. Data are aligned according to circadian phase (X-axis), with corresponding approximate clock time shown on the top X-axis. 0° represents core body temperature minimum (~5 AM in these subjects). Thin gray shaded bars are shown at the top of each panel to indicate the average equivalent clock time when subjects would normally sleep when at home (although all data were collected during wakefulness in the laboratory). Solid lines represent the Cosinor model fits for each protocol (black lines, constant routine [CR]; green lines, 28-h forced desynchrony [FD28]; red lines, 20-h forced desynchrony [FD20]). Only Cosinor fits that are significant (P<0.05) are shown. The phases of the peak SBP rhythms are shown for each protocol by the respective colored dashed vertical lines. There was no urine flow data for FD20. Catecholamines were assayed from urine samples in the CR and FD28 and from plasma in the FD20. In all three protocols, SBP rhythmicity had very similar peak to trough amplitudes (3-6 mmHg) and almost identical phases of circadian peaks (average 244°; equivalent to ~9 PM), which was similar to the timing of the peaks in DBP, but quite distinct from the circadian peaks in catecholamines, cortisol and heart rate, and quite distinct from the troughs in urine excretion and cardiac vagal modulation (see Supplemental Table I for numerical phases).