Endogenous Circadian Rhythm in Vasovagal Response to Head-Up Tilt

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Background—The incidence of syncope exhibits a daily pattern with more occurrences in the morning, possibly as a result of influences from the endogenous circadian system and/or the daily pattern of behavioral/emotional stimuli. This study tested the hypothesis that the circadian system modulates cardiovascular responses to postural stress, leading to increased susceptibility to syncope at specific times of day.

Methods and Results—Twelve subjects underwent a 13-day in-laboratory protocol in which subjects’ sleep-wake cycles were adjusted to 20 hours for 12 cycles. A 15-minute tilt-table test (60° head-up) was performed ~4.5 hours after scheduled awakening in each cycle so that 12 tests in each subject were distributed evenly across the circadian cycle. Of 144 tests, signs/symptoms of presyncope were observed in 21 tests in 6 subjects. These presyncope events displayed a clear circadian rhythm (P=0.028) with almost all cases (17/21) occurring in the half of the circadian cycle corresponding to the biological night (10:30 PM to 10:30 AM). Significant circadian rhythms were also observed in hemodynamic and autonomic function markers (blood pressure, heart rate, epinephrine, norepinephrine, and indices of cardiac vagal tone) that may underlie the circadian rhythm of presyncope susceptibility.

Conclusions—The circadian system affects cardiovascular responses to postural stress, resulting in greater susceptibility to presyncope during the night. This finding suggests that night-shift workers and people with disrupted sleep at night may have greater risk of syncope as a result of their exposure to postural stress during the biological night. (Circulation. 2011;123:961-970.)

Key Words: cardiovascular response ■ circadian rhythm ■ syncope

Syncope is a sudden and transient loss of consciousness caused by transient global cerebral hypoperfusion. A common presenting problem in healthcare settings, syncope accounts for ~1% of emergency room visits. Syncope may cause major morbidity such as fractures and motor vehicle accidents (~6% of patients) and minor injuries such as laceration and bruises (~29% of patients). The impacts of syncope on public safety (eg, syncope while driving) have also attracted recent interest.

Clinical Perspective on p 970

The most common type of syncope is vasovagal syncope (VVS), which is associated with hypotension and/or bradycardia and is neurally mediated by vagal excess and sympathetic withdrawal. VVS accounts for >77% of reported syncope episodes and occurs more frequently in young individuals. The incidence of VVS displays a daily pattern with a broad peak in the morning (6 AM to noon). The peak conceivably could be caused by the day/night distribution of behavioral stressors that may trigger VVS such as standing up in the morning after an overnight sleep with nocturnal diuresis and redistribution of body fluids. Alternatively, the daily pattern of syncope could be influenced by the circadian pacemaker that coordinates/generates endogenous rhythms of ~24 hours in numerous neurophysiological processes, including cardiac autonomic function, possibly making the cardiovascular system more vulnerable to stressors at specific circadian times. Hossmann et al explored this possibility by examining sympathetic and adrenergic vascular responses of 5 healthy subjects every 3 hours across a 24-hour period. Their study clearly demonstrated 24-hour rhythms in sympathetic responses to tilt with greater responses at 9 AM and 9 PM. However, the observed rhythms were likely a combined effect of the endogenous circadian cycle and the sleep-wake cycle because these rhythms are usually synchronized and because the subjects were allowed to sleep between 11 PM and 7 AM, were awakened only ~5 minutes before each test so that the influences of sleep or sleep inertia cannot be
excluded. Therefore, the independent influences of the endogenous circadian system on vasovagal response are still unknown.

An important tool for the diagnosis of the VVS susceptibility is the head-up tilt-table test. By introducing orthostatic stress, the tilt-table test is widely used to reproduce syncope in patients susceptible to hypotension and bradycardia. Autonomic cardiovascular regulation plays a major role in the pathogenesis of VVS. We hypothesize that the endogenous circadian system contributes to the daily pattern of vasovagal episodes via influences on the autonomic control, resulting in different physiological responses to the same postural stressor at different circadian times. To test this hypothesis, we examined symptomatic, hemodynamic, and autonomic responses to tilt-table testing at different circadian times (see Methods).

Methods

Subjects

We studied 12 healthy subjects (aged 20 to 42 years, 6 women) who had no history of medical disorders, syncopal attacks, orthostatic hypotension (OH), or impaired autonomic function (see further details in the Methods section in the online-only Data Supplement). Subjects were recruited through advertisements for healthy volunteers in local New England newspapers. To ensure regular oscillations of the circadian pacemaker, we excluded subjects who reported shift work within the prior 3 years or crossing > 1 time zone within the prior 3 months. Additionally, subjects maintained regular sleep-wake cycles with 8-hour sleep opportunities per night for 2 to 3 weeks before undergoing a 13-day in-laboratory protocol. Subjects were free of drugs (including caffeine, alcohol, and nicotine) before and throughout the study. The study was approved by the local Institutional Review Board. Subjects provided informed consent before participation.

Experimental Protocol

To assess endogenous circadian influences on vasovagal responses, we used a “forced desynchrony” protocol with each subject living in a private and constant-temperature laboratory room free of time cues (Figure 1). After 2 baseline days (the same sleep-wake schedule as the preceding 2 to 3 weeks at home), subjects underwent twelve 24-hour sleep-wake cycles with 8-hour sleep opportunities per night for 2 to 3 weeks before undergoing a 13-day in-laboratory protocol. Subjects were free of drugs (including caffeine, alcohol, and nicotine) before and throughout the study. The study was approved by the local Institutional Review Board. Subjects provided informed consent before participation.

Tilt-Table Test

A 15-minute passive head-up tilt test was performed at ~4.5 hours after scheduled waking for each sleep-wake cycle. Before the test, subjects remained in bed in the semirecumbent position (45° upper body) for ~4 hours, during which time the subjects ate breakfast (~3 hours before tilt), rested, and completed computerized questionnaires/tests. Thereafter, subjects were gently slid from the bed to the adjacent tilt table and remained relaxed in the horizontal and supine position throughout a 25-minute baseline period. Then, subjects were tilted to 60° from the horizontal position (head-up with foot plate

Figure 1. Graphical representation of the forced desynchrony protocol. Solid black areas indicate scheduled sleep in darkness (<1 lux); light gray, wakefulness in dim light (~1.8 lux); hatched, wakefulness on baseline days in normal room light (~90 lux); and narrow gray bars, scheduled tilt tests. Subject’s habitual bedtime is 10:00 PM in this example.

support) with a motorized tilting mechanism (model T7605, Metron Medical Australia Pty Ltd, Carrum Downs, Victoria, Australia) and maintained this posture for up to 15 minutes. Note that there was a tilt test during the baseline day so that subjects were partially habituated before the forced desynchrony phase.

The safety procedures involved monitoring: (1) systolic blood pressure (SBP) via an oscillometric arm cuff sphygmomanometer (Dinamap, Critikon Inc, Tampa, FL) at least every 3 minutes during the tilt phase, (2) beat-to-beat SBP changes from finger plethysmography (Portapres, TNO-Biomedical, Amsterdam, Netherlands), (3) ECG for heart rate (HR) and arrhythmias, and (4) symptoms (see Data Acquisition). Tests were aborted whenever any of the following signs and/or symptoms appeared (criteria for presyncope): (1) sustained low SBP of <80 mm Hg (detected via sphygmomanometer) or 15 mm Hg below baseline (excluding cases with stable SBP >100 mm Hg); (2) precipitous SBP decrease of >15 mm Hg in <60 seconds (detected via finger plethysmography), leading to low SBP <80 mm Hg or 15 mm Hg below baseline; (3) HR decrease >20 bpm from baseline (bradycardia) or asystole for ≥5 seconds (no such case occurred); or (4) other symptoms of imminent syncope, including feeling faint, feeling nauseous, experiencing tunnel vision/blacking out, and being unresponsive to questions, or the subject’s request to be tilted down because of these symptoms.

In case of presyncope, the table was rapidly lowered to the horizontal position and the subject was maintained in the supine posture. If vital signs did not normalize immediately or symptoms did not disappear within 30 minutes after tilting down, medical assistance would be called (no such case occurred).

Data Acquisition

Blood pressure (BP) was continuously measured with finger plethysmography. This device cannot accurately estimate absolute levels of brachial artery pressure, and a cross-reference was made to a more accurate automatic oscillometric cuff sphygmomanometer every 5 minutes during baseline and at least every 3 minutes during tilt.

ECG waveforms were continuously recorded at 256 Hz with an ambulatory recording device (Vitaport, Temec Instruments, Kerkrade, the Netherlands) to assess autonomic nervous system activity.

Core body temperature was sampled every minute throughout with a rectal temperature sensor (Yellow Springs Instrument Company, Yellow Springs, OH) to provide a marker of the circadian phase.
Blood was sampled via an indwelling catheter in a forearm vein that was in place throughout the entire study. Only 5 cm$^3$ blood was drawn each time. Epinephrine and norepinephrine were measured, from blood samples collected after 10 minutes of baseline and after 5 to 10 minutes of tilt in each test, with chemiluminescent assays (sensitivity 1 pg/mL and 40 pg/mL for epinephrine and norepinephrine, respectively) as markers of sympathetic activity.

The degrees of nausea, “feeling hot,” and general discomfort were rated separately by subjects at least every 3 minutes during tilt using an 11-point rating scale with 0 indicating no symptoms and 10 indicating extreme symptoms. In each test, the maximum scores were used as indices of the subjective tilt response.

### Data Analysis

To assess tilt responses, cardiovascular variables during baseline and the stable tilt conditions were compared. Data during the first minute after tilting up and the last minute before tilting down were excluded to avoid influences from the postural transitions.

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**Circadian Phase Estimation**

The endogenous circadian cycle affects core body temperature with a period of $\sim$24 hours and thus can be estimated by nonlinear regression of individual core body temperature recordings (Figure I in the online-only Data Supplement). Using the obtained circadian period of each subject (mean, 24.09 hours; range, 23.8 to 24.6 hours) and assigning phase 0° to the time of each subject’s minimum core body temperature (mean, $\sim$4:30 AM), all data were assigned the respective circadian phases (0° to 360°; Figure II in the online-only Data Supplement).

**HR Variability Analysis**

To assess autonomic nervous system activity, the time-domain HR variability analysis was performed. R waves in the ECG were identified with a QRS wave detector based on amplitude and the first derivative of the ECG waveform and were visually scanned by a trained technician to ensure that only normal R waves were included. Mean and SD of normal-to-normal (SDNN) beat intervals were calculated, along with the following indices of cardiac parasympathetic tone: square root of the mean of squares of differences between adjacent
normal-to-normal intervals (RMSSD), and percentage of differences between adjacent normal-to-normal intervals that are >50 ms (pNN50). The frequency-domain HR variability analysis was also performed (Methods and Figures III and IV in the online-only Data Supplement).

BP Analysis
With the use of Beatscope Software (version 1.1, Finapres Medical Systems, Amsterdam, the Netherlands), beat-to-beat SBP, diastolic BP (DBP), HR, stroke volume (SV), cardiac output (CO), ejection time, and total peripheral resistance (TPR) were derived from BP waveforms (finger plethysmography). The low-frequency power of SBP was obtained as an additional index of sympathetic activity changes (Materials and Figures V and VI in the online-only Data Supplement).

Statistical Analysis
All tilt tests for a subject were treated as repeated measures without causal relationship among adjacent tests with the assumption that 20 hours is sufficient for full recovery should presyncope have occurred in a prior test. Four types of statistical analyses were performed. First, to address the primary goal of assessing the circadian distribution of presyncope events, a generalized linear mixed model was used with presyncope as the response (present or absent), circadian bin as a fixed effect (divided into six 60° bins), and subject as a random factor for intercept (Table I in the online-only Data Supplement). In this primary analysis, test outcomes of all subjects (144 tests) were included. Next, in a secondary analysis, subjects were divided into 2 groups: those with presyncope (presynopal group) and those who did not experience presyncope (nonpresynopal group). To assess the effects of tilt, group, and their interactions on continuous physiological variables, mixed-model ANOVAs were performed with subject as a random effect for intercept (Table II in the online-only Data Supplement). Third, similar mixed models were applied to assess different tilt responses between trials with and without presyncope within the presynopal group (72 tests; Table III in the online-only Data Supplement). Finally, cosinor analyses18 using mixed models were applied to test the effects on physiological variables of circadian phase and its interactions with tilt (see Methods and Table IV in the online-only Data Supplement). Actual circadian phases of data (instead of 60° bins) were used in the cosinor analyses.

Results
Presyncope During Tilt-Table Testing
Twenty-one cases of presyncope occurred in 6 subjects. In all 21 cases, signs/symptoms of presyncope disappeared almost instantaneously after tilting down, and BP and subjective symptoms normalized within 3 minutes after tilting down. No cases of fully developed syncope occurred, presumably because the tests were aborted when clear signs/symptoms of presyncope appeared. Figure 2 shows a typical presyncope event that is clearly a vasovagal event, indicated by a delayed and precipitous decrease in SBP occurring after \( \sim \)12 minutes of tilt, along with bradycardia at the end of the test. In other presyncope events, the phase of reflex bradycardia and/or vasodepressor effects accompanying VVS were not always as pronounced because we aborted the tests early to avoid syncope and to reduce the burden on the volunteers during this prolonged and intensive study. In addition to the SBP decrease, significant falls in SV and CO, without a fall in TPR, were observed just before presyncope occurred (Figure XII in the online-only Data Supplement).

Endogenous Circadian Rhythm in Presyncope Events
The 21 presyncope events did not occur randomly across the circadian cycle but displayed a significant circadian rhythm (generalized linear mixed model, \( P=0.028 \); Figure 3B) with a peak at the circadian phase bin centered around 0° (corresponding to \( \sim \)4:30 AM). Figure 3A shows an example subject with tilt tests aborted consistently during the biological night,
Figure 3B shows the group average probability of presyncope occurrence at different circadian phases. From the model output, the mean probability across the biological night and early morning (270° to 90°; corresponding to 10:30 PM to 10:30 AM) is 16.8%, 9 times the probability from the other half of the circadian cycle (Figure 3B). There was no training effect on presyncope occurrences throughout the protocol (11 presyncope events in the first 6 cycles and 10 in the last 6 cycles). The distribution of presyncope events at different cycles confirmed a strong circadian influence indicated by 2 peaks located at cycles 3 and 9 when tilt tests were performed during the biological night (Figure VII in the online-only Data Supplement).

**Classification of Presyncope Events**

The presyncope events could be divided into 2 categories. In the first category, 17 cases were associated with a precipitous SBP drop (detected with finger plethysmography) leading to 15 mm Hg below baseline (detected with sphygmomanometry; 4 cases), SBP <80 mm Hg alone (1 case), or both (12 cases). Sphygmomanometric SBP within 2 minutes before tilting down was 81.1 ± 5.1 (mean ± SE) mm Hg in these 17 trials (baseline SBP, 99.8 ± 3.2 mm Hg). Twelve of these 17 cases (including the one with only SBP <80 mm Hg) were also associated with symptoms of imminent syncope (criterion 4). In the second category, 4 cases were associated with only symptoms of imminent syncope without hypotension, ie, SBP of 89.0 ± 4.6 mm Hg when being tilted down (baseline SBP, 97.4 ± 2.1 mm Hg). No presyncope cases were based on a sustained low SBP (15 mm Hg below baseline and <100 mm Hg) (criterion 1). No cases of serious arrhythmias or asystole occurred.

Both VVS and syncope resulting from OH can be triggered by orthostatic stress, and their manifestations often overlap. We classified all 21 presyncope events as vasovagal presyncope rather than OH for the following reasons. First, from a pathophysiological point of view, the difference between VVS and OH is that OH is due to autonomic function failure \(^1\) and VVS is due to intermittently inappropriate cardiovascular reflexes in response to a trigger. Here, all subjects were healthy adults without a history of OH or impaired autonomic function. Thus,
OH was not expected in this group. Second, all presyncope events occurred after >4 minutes of tilt (mean±SE, 10.7±0.6 minutes; Figure VIII in the online-only Data Supplement). This is different from classic OH-induced syncope/presyncope that occurs within 3 minutes of standing or tilt. Third, delayed OH can cause syncope/presyncope after 3 minutes of tilt. However, delayed OH is characterized by progressive decreases of SBP and TPR after tilt-up, whereas none of 21 cases in the current study fit such a description of delayed OH.

**Cardiovascular Responses to Head-Up Tilt**

To determine whether there were any underlying physiological differences that could explain the sporadic occurrence of presyncope, comparisons were performed between nonpresyncope and presyncope subjects and between the 21 presyncope trials and the 51 trials without presyncope in presyncope subjects. The following results reflect only the responses during the stable tilt period.

**General Effects of Head-Up Tilt**

In responses to tilt, subjects showed decreased SBP, increased DBP, increased HR, increased sympathetic activity (epinephrine and norepinephrine levels), and decreased vagal tone (SDNN, RMSSD, and pNN50; Figure 4). The tilt effects on autonomic nervous activity were confirmed by the frequency-domain HR variability analysis and the SBP spectral analysis (Figures III and V in the online-only Data Supplement).

**Group Differences**

Presyncope subjects had reduced tilt responses in all variables except epinephrine and HR compared with the nonpresyncope subjects (see the $P$ values for tilt by group in Figure 4). There were no significant differences in group means of all variables except that SBP was lower in the presyncope group.

**Physiological Responses in Presyncope Trials**

Within the presyncope subjects, the SBP drop during the presyncope trials was larger than that during the trials without
presyncope (Figure 5A). The tilt-induced increases in DBP and norepinephrine were less in the presyncope trials although the reductions did not reach significance (DBP: mixed-model ANOVA, $P = 0.05$; norepinephrine: $P = 0.08$; Figure 5D and 5E).

**Circadian Rhythmicity**

There were overall (both conditions) significant circadian rhythms in all reported physiological variables: SBP, HR, epinephrine, and norepinephrine were lowest and cardiac parasympathetic markers (SDNN, pNN50, and RMSSD) were highest during the biological night (28° to 45° or ≈11:30 PM to 7:30 AM; Figure 6 and the Table). The circadian influences were smaller than the tilt effects on all variables except SBP, which yielded similarly sized effects.

During the biological night, the tilt-induced epinephrine increase was smaller and the tilt-induced decreases in
Circadian Rhythms

We found in healthy subjects that the susceptibility to presyncope due to head-up tilt was much higher during circadian phases corresponding to the biological night (equivalent to 10:30 PM to 10:30 AM in these subjects). The symptoms of nausea and general discomfort during tilt also became worse across the biological night. These findings provide strong evidence that the circadian system modulates vasovagal responses, yielding different responses to the same stressor at different circadian times. We also found significant circadian rhythms in indices of hemodynamics and autonomic activity such as SBP, DBP, HR, epinephrine, norepinephrine, and HR variability–derived parasympathetic markers that likely underlie the circadian modulation of vasovagal responses.

Table. Peak/Trough Phase Locations and Amplitudes of Circadian Rhythms

<table>
<thead>
<tr>
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<th>Baseline</th>
<th>Tilt</th>
<th>(Circadian)</th>
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</thead>
<tbody>
<tr>
<td>SBP, mm Hg</td>
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<td></td>
</tr>
<tr>
<td>Peak phase</td>
<td>231.7±11.8°</td>
<td>254.1±9.6°</td>
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<tr>
<td>Trough phase</td>
<td>340.7±15.2°</td>
<td>357.7±9.4°</td>
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<tr>
<td>Peak-to-trough amplitude</td>
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<tr>
<td>Tilt effect</td>
<td>−2.6±0.6</td>
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<tr>
<td>DBP, mm Hg</td>
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<td></td>
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<tr>
<td>Peak phase</td>
<td>233.3±26.4°</td>
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<tr>
<td>Trough phase</td>
<td>119.2±27.4°</td>
<td>173.6±10.6°</td>
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<tr>
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<tr>
<td>Tilt effect</td>
<td>9.3±0.4</td>
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<td>HR, bpm</td>
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<td>189.8±24.4°</td>
<td>177.0±10.9°</td>
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<tr>
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<td>Peak-to-trough amplitude</td>
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<td>&lt;0.0001</td>
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<tr>
<td>Tilt effect</td>
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<td>Epinephrine, pg/mL</td>
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<tr>
<td>Peak-to-trough amplitude</td>
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<tr>
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<tr>
<td>Norepinephrine, pg/mL</td>
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<td>Peak phase</td>
<td>120.9±28.9°</td>
<td>202.3±13.4°</td>
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<tr>
<td>Trough phase</td>
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<tr>
<td>Peak-to-trough amplitude</td>
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<tr>
<td>Tilt effect</td>
<td>163.9±8.7</td>
<td></td>
<td></td>
</tr>
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<td>SDNN, ms</td>
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<td>35.6±8.9°</td>
<td>345.9±19.0°</td>
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<tr>
<td>Trough phase</td>
<td>265.0±21.4°</td>
<td>228.4±14.1°</td>
<td></td>
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<tr>
<td>Peak-to-trough amplitude</td>
<td>25.3±4.8</td>
<td>15.2±5.0</td>
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<tr>
<td>Tilt effect</td>
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<tr>
<td>RMSSD, ms</td>
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<td></td>
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</tr>
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<td>8.8±25.3°</td>
<td>113.8±26.6°</td>
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<tr>
<td>Trough phase</td>
<td>203.6±29.7°</td>
<td>221.7±21.1°</td>
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<tr>
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<td>8.6±5.0</td>
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<tr>
<td>Tilt effect</td>
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<tr>
<td>pNN50, %</td>
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<td></td>
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<tr>
<td>Peak phase</td>
<td>5.7±69.1°</td>
<td>96.8±48.5°</td>
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<tr>
<td>Trough phase</td>
<td>188.3±21.7°</td>
<td>220±43.7°</td>
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<tr>
<td>Peak-to-trough amplitude</td>
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<tr>
<td>Tilt effect</td>
<td>−31.9±1.4</td>
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Data are presented as mean±SE. Results were calculated from the cosinor fits (Figure 6) obtained from the cosinor analyses using the mixed-model ANOVAs (Table VI in the online-only Data Supplement). P values for circadian effects are based on combined baseline and tilt data.

RMSDD and pNN50 were larger than during the biological day (Figure 6). For instance, the epinephrine increase at 330° (≈15.0 pg/mL) was much smaller than the increase at 200° (≈33.1 pg/mL).

Subjective Responses to Head-Up Tilt

A different subjective scoring system was used in the first 2 subjects (1 presyncopal and 1 nonpresyncopal), so those results were excluded from the group analyses of subjective tilt responses. There were no group mean differences between presyncopal and nonpresyncopal subjects in their maximum scores of nausea, feeling hot, and general discomfort (Figure XIII in the online-only Data Supplement). Within the presyncopal subjects, all subjective responses were significantly higher when presyncope occurred: nausea, 0.9±0.5 (mean±SE) without presyncope versus 4.6±0.6 for presyncope trials; feeling hot, 1.6±0.5 versus 4.1±0.6, respectively; general discomfort, 1.1±0.5 versus 4.6±0.6, respectively (mixed-model ANOVA, P<0.0001 for all 3 measures). Significant circadian rhythms were observed in all subjective measures with a trough at 130° to 200° (~1:10 to 5:50 AM) and a peak at 340° to 10° (~3:10 to 5:10 AM). These circadian rhythms showed no significant group differences except that nausea displayed a larger circadian variation in the presynopal group (cosinor analyses, P=0.0017; Figure XIII in the online-only Data Supplement).

Discussion

BP Regulation During Tilt-Table Testing

Arterial BP while upright is maintained predominantly through the regulation of the sympathetic outflow, leading to increased HR, cardiac contractility, and peripheral vasconstriction. We found that SBP was lower in presyncopal subjects than nonpresyncopal subjects (Figure 4A), suggesting a threshold effect whereby lower baseline SBP is more likely to be associated with presyncope. Moreover, SBP is lower during the biological night, thereby increasing the likelihood of presyncope at that time. A previous study suggested that a steep fall in CO is the main mechanism in the initiation of a vasovagal faint.21 Our study supports such a CO-mediated mechanism for the initiation of hypotension because CO and SV decreased significantly while TPR showed no significant decrease before tilt-down before the occurrences of presyncope (Figure XII in the online-only Data Supplement).

Changes in neurohumoral factors can be important mechanisms underlying development of syncope/presyncope dur-
ing head-up tilt. As demonstrated in this study, circadian influences on epinephrine and norepinephrine may contribute to the observed circadian rhythm of presyncope, eg, lower epinephrine during the biological night when presyncope occurred more frequently. The mechanistic link between endogenous circadian rhythms of neurohumoral factors and presyncope is yet to be elucidated.

Tilt-Table Test Reproducibility
The head-up tilt-table test is widely used to diagnose VVS.\(^1\) However, reproducibility of the test is still a concern. The long-term reproducibility of positive tilt responses (with presyncope/syncope) varies from 62% to 85%,\(^2\) and 1-day reproducibility may be as low as 35%.\(^3\) The present study likely represents one of the best-controlled tilt-table data sets because all tests were performed at the same time after waking up, with all scheduled events rigorously controlled for all test cycles. We showed that the circadian time when tests are performed is a very important source of variation; eg, the chance of experiencing presyncope at the circadian phase 0° (=4:30 AM) was >20 times larger than that at 180° (=4:30 PM). Thus, nocturnal tilt tests could potentially be a sensitive method to reveal individuals at higher risks for syncope. The observed presyncope events might be interpreted as “false-positive” responses because these subjects had no history of syncope. However, other studies have suggested that vasovagal susceptibility is probably present in all healthy humans.\(^4\) Thus, it is possible that these subjects might be asymptomatic mostly because they have never previously experienced orthostatic stress during nighttime.

Limitations
Although an endogenous circadian rhythm in presyncope susceptibility is very clear, there are certain limitations regarding the underlying mechanism. First, we studied healthy young subjects without a history of syncope. Although VVS can occur in young and ostensibly healthy people, it is important to validate our findings in populations more susceptible to VVS. Second, the underlying mechanism causing presyncope/syncope may be different during passive postural changes (as in this study) compared with standing up actively when the leg muscle contractions help maintain venous return and SBP.\(^5\) Third, the 4 presyncope events based on only symptoms of imminent syncope without hypotension might not have developed into syncope. However, the circadian rhythm of presyncope events remains after exclusion of the 4 cases (Figure IX in the online-only Data Supplement). Fourth, it is conceivable that more presyncope/syncope events would have occurred if tilt were extended beyond 15 minutes. However, the peak of presyncope incidence occurred at 11 to 12 minutes of tilt (Figure VIII in the online-only Data Supplement). Thus, the choice of the maximum tilt duration is unlikely to have affected the observed circadian rhythm of presyncope events. Fifth, among our criteria for presyncope, using a relatively arbitrary absolute SBP threshold for aborting the tilt test (criterion 2, SBP <80 mm Hg) could artificially introduce more aborted tests when baseline SBP was lower. However, this is unlikely because only 1 presyncope event was based on this threshold criterion alone, and this 1 case did not occur in the biological night. Additionally, the subjective responses during the stable tilt condition displayed circadian rhythms with greater responses at the circadian time corresponding to the peak of presyncope occurrences. Thus, the circadian rhythm of presyncope distribution is likely to reflect a real effect of the circadian system on VVS susceptibility.

Potential Implications
This study provides direct evidence that the circadian pacemaker influences vasovagal responses to head-up tilt, leading to higher susceptibility to presyncope overnight. Such a vulnerable time window may not be a concern for people with normal sleep-wake schedules who would sleep through the vulnerable period without exposure to postural stressors. However, the vulnerable time window may have a greater impact on individuals who have to remain awake or wake up during the nighttime such as shift workers, military personnel, emergency workers, airline pilots, truck drivers, parents of infants, and people with nocturia, insomnia, or other sleep disorders. Such populations may be at a higher risk for syncope, which could have important consequences on personal and public safety.

Although most syncope events occur during the daytime, previous studies also documented VVS during the normal hours of sleep (eg, 10 PM to 7 AM) in nonintoxicated adults who wake up feeling faint and may briefly lose consciousness in bed or immediately on standing.\(^6\) The occurrence of such nocturnal syncope (called sleep syncope) has been a puzzle. Jardine et al\(^7\) hypothesized that sleep syncope may be linked to different cardiovascular responses to afferent stimuli during sleep (eg, the centrally mediated increase in vagal activity during the deeper phases of non–rapid-eye-movement sleep). Our finding of the highest presyncope risk at ~4:30 AM mediated by the circadian system (Figure 3B) suggests that the endogenous circadian system also may be mechanistically involved in the pathophysiology of sleep syncope. The possible underlying mechanisms include the observed circadian-modulated increase in parasympathetic nervous activity, decreased sympathetic nervous system activity, decreased SBP (Figure 6), and decreased CO (Figure XI in the online-only Data Supplement) during the biological night.

Acknowledgment
We thank Dr Wei Wang for statistical consultation.

Sources of Funding
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Disclosures
None.

References

CLINICAL PERSPECTIVE
Vasovagal syncope, the most common type of syncope, displays a daily pattern with more occurrences during the morning (6 AM to noon). This pattern could be caused by the daily distribution of behavioral/emotional stimuli and/or modulation of physiological responses by the endogenous circadian system (“body clock”). The present study provides strong evidence that the circadian system could contribute to the daily pattern of vasovagal syncope via its influences on hemodynamic and autonomic responses to tilt stressor. We found that the vulnerability to presyncope caused by head-up tilt has a strong endogenous circadian rhythm, with susceptibility 9 times greater at the circadian times between 10:30 AM and 10:30 PM compared with between 10:30 AM and 10:30 PM. This finding highlights the importance of performing tilt-table tests at similar circadian times when comparing responses of different individuals or the same person before and after treatments for syncope. Additionally, a higher sensitivity may be achieved by performing tilt-table testing during early morning hours or the nighttime. The identified vulnerable period may have relevance to individuals who remain awake or wake up frequently during the nighttime such as night-shift workers, parents feeding their infants, and elderly people with increased nocturia and insomnia. These people may be at higher risk for syncope as a result of their exposure to postural stress during the nighttime. Moreover, the morning broad peak of vasovagal syncope observed in the epidemiological studies might be a combined effect of the endogenous circadian system and daily patterns of external behavioral stimuli.
Endogenous Circadian Rhythm in Vasovagal Response to Head-Up Tilt
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SUPPLEMENTAL MATERIAL

Supplemental Methods

Subject recruitment

All subjects were recruited by putting advertisements for healthy volunteers in local New England newspapers. Thereafter, candidates underwent a phone screen to check initial eligibility. The health status of those potential subjects who satisfied the inclusion criteria and did not meet any exclusion criteria, and who were available and willing to undertake a 13 day in-laboratory study was then confirmed by extensive medical history questionnaires followed by electrocardiography, blood chemistry profiles, liver function tests, complete blood count, urinalysis, a history and physical examination (plus a sit-to-stand orthostatic challenge test) by a physician, and a psychiatric and psychological examination by a clinical psychologist. Subjects were excluded if they had history of impaired autonomic function, syncopal attacks, or orthostatic hypotension; or if they were obese (body mass index >30), taking any medications, or had any chronic or current acute medical or psychiatric disease including depression or other psychopathology (e.g., Beck Depression Inventory score ≥10 or Minnesota Multiphasic Personality Inventory score ≥ 90), hypertension, anemia or other hematologic, hepatic abnormalities; if they reported any current or chronic sleep disturbances or sleep disorders, recent shift work, or circadian rhythm disturbances; if they had a first-degree relative with psychiatric history; or if they reported substance abuse, caffeine dependence (> 4 caffeinated beverages per day), alcohol dependence (> 14 alcoholic drinks per week), or nicotine dependence (> 4 cigarettes per day). Finally, use of all of these substances had to be stopped 3 weeks prior to the start of the inpatient study. To verify these self-reports, a urine sample was tested early in the screening process and at admission to the in-laboratory phase of the study to confirm that the subjects were free from drugs of abuse, alcohol,
caffeine, and nicotine. This recruiting procedure and the study protocol were approved by the local Internal Review Board. All subjects provided informed consent prior to the study.

**Circadian analysis**

The sleep-wake (or rest-activity) cycle affects core body temperature (CBT) as does the endogenous circadian cycle controlled by the circadian pacemaker\(^1\). Normally (i.e., outside the laboratory) the sleep-wake cycle and the circadian cycle in humans are ‘entrained’ with the same period (24 hours), together causing a reduced CBT during sleep at night. In the forced desynchrony (FD) protocol, the two rhythms have different periods, i.e., 20 hours for the imposed behavioral (sleep-wake) cycle and \(~24\) hours for the endogenous circadian cycle. Thus, the behavioral and circadian rhythms oscillate back and forth between in-phase and out-of-phase states gradually throughout the FD, and the phase relationship between the two rhythms oscillates with a period of 5 days (six 20-hour cycles). For instance, during the first and 7\(^{th}\) behavioral cycles, the behavioral cycle and the circadian cycle were in phase (sleep during the biological night), causing a higher amplitude of the overall CBT rhythm (see 0-24 hours and 120-144 hours in **Supplemental Figure 1**); and on other days sleeping occurs during (some of) the biological day reducing CBT when the circadian cycle is increasing CBT, resulting in a reduced CBT amplitude. With knowledge of the imposed behavioral cycle (20 hours), the phase, period and amplitude of the underlying circadian CBT rhythm can be statistically estimated by nonlinear least squares regression, which is a standard technique in the circadian field\(^1\). The components in the regression include multiple sinusoidal waveforms to represent (1) the fundamental and additional 7 harmonics of the 20-hour behavioral cycle (periods = 20, 20/2, 20/3, …, 20/8 hours, respectively), and (2) the fundamental and second harmonics of the circadian rhythm (periods = \(~24\), \(~24/2\) hours, respectively). A polynomial trend was also included if there is any significant effect across
all days of the protocol. In the current study, the mean circadian period (the fundamental circadian waveform) was 24.09 h [range 23.8-24.6 h] in these subjects (Supplemental Figure II).

Circadian phases are expressed in degrees, with 360° representing the period of the circadian oscillator (~24 h). For each subject, each data point was assigned a circadian phase (between 0° and 360°) depending upon the time of the point from the CBT minimum of the fitted circadian waveform (0°) and the subject’s estimated circadian period (one full circadian period = 360°) (Supplemental Figure II). To assess the circadian rhythms of cardiovascular variables, we performed cosinor analyses in which actual phases for data (instead of 60° phase bins) were used to yield the cosinor model plots (e.g., see lines on Figure 6 and Supplemental Figure IV). In the cosinor analysis of each cardiovascular variable, we included both the fundamental (~24-h) and second harmonics (~12-h) of the endogenous circadian rhythm to allow fitting of a more complex circadian oscillation instead of a simple sinusoidal waveform (see Cosinor analysis using mixed model ANOVA below).

In order to aid understanding and relevance to living outside the laboratory, these circadian phases (0-360°) are also presented as equivalent clock time across the day (0-24 hour). Note, for this calculation, it is necessary to determine the clock time at which the first CBT minimum occurred when entering the lab (Supplemental Figure II). Although no binning was used in the cosinor analyses, for visualization purposes and to provide evidence that the model fits the data, it is appropriate to present data points superimposed on the model fit. However, since data were not obtained at every single phase, and since different subjects have data at slightly different phases from each other, the data were averaged into the smallest bin whereby all subjects contribute data to each bin. This optimal bin width for this protocol was 60°, which approximates into ~4 h per bin.
Cosinor analysis using mixed model ANOVA

To assess circadian rhythms, Halberg initially provided a simple cosinor model which fits repeated observations to a fundamental sinusoidal regression function 2:

\[ Y_i = \mu + \alpha \cos(2\pi \frac{t_i}{\tau} - \beta) + \epsilon_i \]  

(1)

where \( Y_i \) is the \( i \)th observation (or data point) at time \( t_i \), \( \mu \) is the mean or MESOR (midline estimating statistic of rhythmicity), \( \tau \) is the period of the rhythm, \( \alpha \) is the amplitude, \( \beta \) is the acrophase, and \( \epsilon \) is the residual error. \( \mu, \alpha \) and \( \beta \) are the three parameters to be determined from the best fit of all data points \( \{t_i, Y_i\} \). This model in Eq. (1) was later extended to incorporate rhythms of multiple periodicities or non-sinusoidal waveforms and to account for random effects 3-5:

\[ Y_i = \mu + \sum_{k=1}^{h} \alpha_k \cos(2\pi \frac{t_i}{\tau_k} - \beta_k) + \epsilon_i \]

(2)

where the index \( k \) indicates the \( k \)th rhythm or harmonic, and \( h \) indicates the total number of sinusoidal functions with different periods. The cosinor model is a nonlinear model in the amplitude and acrophase parameters of sinusoidal functions and it can be transformed to a linear regression model:

\[ Y_i = \mu + \sum_{k=1}^{h} [a_k f_k(t_i) + b_k g_k(t_i)] + \epsilon_i \]

(3)

where the functions \( f_k(t_i) = \cos(2\pi \frac{t_i}{\tau_k}) \) and \( g_k(t_i) = \sin(2\pi \frac{t_i}{\tau_k}) \) represent the transformation, and the original amplitudes and acrophases can be computed from the transformed linear coefficients \( a_k \) and \( b_k \) using the following equations:

\[ \alpha_i = \sqrt{a_k^2 + b_k^2} \quad \beta_k = \tan^{-1}(b_k / a_k) \]

(4)

To determine whether or not there are significant circadian rhythms of our physiological variables of interest (e.g., SBP, DBP and HR), we adopted and modified the cosinor model as
described in Eq. (3). Instead of using time \( t_i \) and period \( \tau_k \), we used circadian phase relative to CBT minimum \( \theta_i \) in order to combine data points from different individuals while accounting individual differences in the circadian period (see Supplemental Figure II). To better describe circadian rhythms of non-sinusoidal shapes, we included the fundamental rhythm of ~12 hours and its first harmonic [i.e., \( h=2 \) in Eq. (3)], which are generally sufficient for adequate description of most physiological circadian rhythms. Moreover, we considered additional effects of condition (baseline or tilt), and its interactions with the four circadian terms. Thus, the full cosinor model used in our study can be described by the following equation:

\[
Y_i = \mu + a_1 \cos(\theta_i) + b_1 \sin(\theta_i) + a_2 \cos(2\theta_i) + b_2 \sin(2\theta_i) + \left[ c_0 + c_1 \cos(\theta_i) + c_2 \sin(\theta_i) + c_3 \cos(2\theta_i) + c_4 \sin(2\theta_i) \right] * C + \varepsilon_i
\]

where \( C \) is the binary variable for condition (baseline or tilt). There are total 10 coefficients in the model (including intercept) to be determined using multiple linear regressions.

To estimate the coefficients in Eq. (5) for each physiological variable, we pooled all data points of all subjects together and performed a mixed model ANOVA using standard least square regression and the restricted maximum likelihood method (JMP 8.0, SAS Institute Inc, North Carolina). In the mixed model, the terms in Eq. (5) (except for \( \varepsilon_i \)) were included as fixed effects and subject as a random effect for intercept (see Detailed Model information in Table IV). The resultant \( p < 0.05 \) for either \( \cos \theta \) or \( \sin \theta \) indicates a significant circadian rhythm of ~24 hours (the lower of the two \( p \) values is always reported in the results). Similarly, \( p < 0.05 \) for either \( \cos 2\theta \) or \( \sin 2\theta \) indicates a significant harmonic rhythm (~12 hours). Based on the obtained regression coefficients and their standard errors from the mixed model, we then calculated the phase locations of the overall peak, the overall trough, the peak-to-trough amplitude, and their standard errors.
Supplemental Results

Heart rate variability analysis in the frequency domain

In addition to the heart rate variability (HRV) analysis in the time domain, we performed the HRV analysis in the frequency domain according to the published standards. Briefly, normal-to-normal heart beat intervals (R-R intervals from adjacent EKG waveforms) were re-sampled to 3.41 Hz (1024 points every 300 seconds) using cubic spline fitting; the power spectrum of R-R intervals was obtained in each 2.5-minute window without gaps of data more than 5 seconds; and the average power spectrum was obtained from all 2.5-minute windows at baseline or during tilting for each cycle. Four additional HRV indices are presented (Supplemental Figures III and IV):

(i) Total spectral power (TP) of heart rate fluctuations at <0.4Hz. It is known that TP is highly correlated to SDNN. Using a simple linear regression, we found a strong association between TP and SDNN (r = 0.89, p < 0.0001).

(ii) High frequency power (HF: 0.15-0.4 Hz). Log scale of HF (lnHF) is used to ensure a normal distribution. As a parasympathetic marker, lnHF is highly correlated to RMSSD and pNN50 which are also parasympathetic markers. Using data obtained in this study, we confirmed that there were strong associations between lnHF and RMSSD (r = 0.90, p < 0.0001) and between lnHF and pNN50 (r = 0.88, p < 0.0001).

(iii) Low frequency power (LF: 0.04-0.15 Hz). Log scale of LF (lnLF) is used to ensure a normal distribution. lnLF is contributed by both sympathetic and parasympathetic nervous activities and, thus, is not considered as a reliable sympathetic or parasympathetic marker.

(iv) Ratio of LF and HF powers (LF/HF). LF/HF generally reflects the balance of sympathetic and parasympathetic activities, i.e., large values indicate relatively strong
sympathetic activity while small values indicate relative strong parasympathetic activity.

Similar to the results of the time-domain HRV analysis, the frequency-domain HRV analysis revealed that heart rate variability (TP) and parasympathetic indices (lnHF) significantly decreased in response to tilt stress for both groups (Supplemental Figures IIIA and IIIB). We also found that LF/HF increased in response to tilt stress (Supplemental Figure IIID). These findings are consistent with the observation that sympathetic nervous activity assessed by plasma epinephrine and norepinephrine levels increased in response to tilt (Figures 4C and 4D). The tilt-induced decrease in TP was smaller in the subjects with presyncope (P = 0.0008; Supplemental Figure IIIA) and there were no significant group differences in the tilt effects on other frequency-domain HRV indices (Supplemental Figures III B-III D). lnLF showed no significant changes in response to tilt except for the trials of presyncope in which lnLF decreased (P = 0.03) (Supplemental Figure IIIC). Comparing the 21 trials with presyncope and the 51 trials without presyncope within the same 6 presyncopal subjects, only lnHF showed significantly different tilt-induced change, i.e., the decrease of lnHF was greater during the trials with presyncope (P = 0.003) (Supplemental Figure III B).

TP, lnHF, and lnLF showed significant circadian rhythms (Supplemental Figure IV). All these circadian rhythms showed no significant group differences (P > 0.1 for all variables). Note that the circadian profile of TP was virtually identical to that of SDNN (Figure 6F) and that the circadian profile of lnHF was virtually identically to that of RMSSD (Figure 6G), e.g., for all four variables, the peak was at ~40° at baseline and the valley was at ~240° during tilt. LF/HF and its response to tilt showed no significant circadian rhythms.
Power spectral analysis of systolic blood pressure

It has been proposed that the low-frequency (LF, 0.04-0.15Hz) oscillations in systolic blood pressure (SBP) can be used as a marker to monitor changes of sympathetic activity\textsuperscript{7-9} though certain studies indicated controversial results\textsuperscript{10,11}. Using SBP recordings measured from finger plethysmography, we obtained low frequency power of SBP fluctuations. Briefly, SBP signals were re-sampled to 3.41 Hz (1024 points every 300 seconds) using cubic spline fitting; the power spectrum was obtained in each 2.5-minute window without gaps of data more than 5 seconds; and the average power spectrum was obtained from all 2.5-minute windows at baseline or during tilting for each cycle.

In addition to SBP LF power, we obtained high frequency power (HF; 0.15-0.5Hz) of SBP which has been suggested to reflect the effect of respiration\textsuperscript{9}. Log scale LF (lnLF) and log scale HF (lnHF) were used in the analyses to ensure the normal distributions of the two variables.

In response to head-up tilt, there was a significant increase in lnLF of SBP (\textit{Supplemental Figure V A}). This finding is consistent with the observation of increased plasma epinephrine and norepinephrine levels during head-up tilt (\textit{Figures 4C} and \textit{4D}), together indicating an increase in sympathetic activity during tilt. The lnLF change was not significant different between groups and between the trials with presyncope and without presyncope (P > 0.1). Similar to the change in epinephrine, the tilt-induced increase in lnLF of SBP showed a significant circadian rhythm with smaller increase during the biological night compared to the biological day (\textit{Supplemental Figure VIA}), i.e., the increase was smallest at ~20° (corresponding to 5:50AM) and largest at ~170° (3:50PM). However, the circadian rhythm in the overall SBP lnLF (both conditions both groups) was not statistically significant.

lnHF of SBP also increased in response to head-up tilt (\textit{Supplemental Figure V B}), suggesting increased respiratory effect during tilt compared to baseline. Mean lnHF and its
response to tilt showed no group differences and no differences between the 21 trials with presyncope and the 51 completed trials for the same presyncope group (P > 0.1). Moreover, mean lnHF and its response to tilt showed no significant rhythms (Supplemental Figure VI B).

Changes in cardiovascular variables throughout the head-up tilt test

Stable phase during head-up tilt. During the stable phase after tilt-up (1-minute period after ~1-minute tilt-up), there was a large decrease in stroke volume (SV) and a significant decrease in ejection time (EJT) for both presyncopal and non-presyncopal groups compared to baseline (Panels A and C of Supplemental Figure X). Cardiac output (CO) also decreased significantly but not dramatically as SV (Supplemental Figure X B) due to increased heart rate (HR) (Mean±SE: 85.5 ± 2.5; baseline 62.1 ± 2.5; P < 0.0001). Total peripheral resistance (TPR) increased at such initial stage of tilt as compared to baseline (Supplemental Figure X D). The decrease in CO and the increase in TPR were more pronounced in the presyncopal group, but the group difference was only significant in the TPR change (the group difference in the CO change did not reach significant level; P = 0.06). Changes of SV and EJT were not significantly different between groups. Within presyncope group, there were no differences in all four variables and their tilt-induced changes between aborted tests and completed tests.

The circadian pacemaker had a significant influence on CO, leading to a significant circadian rhythm (P = 0.00039) with the minimal CO at ~300° (corresponding to ~10:30PM) that was consistent for both groups and during both baseline and tilt conditions (Supplemental Figure XI B). There was also a significant circadian rhythm in EJT but not in SV and TPR (Supplemental Figure XI). There were no interactions between tilt and circadian influences in the four variables. Though average CO was not significantly different between groups (Supplemental Figure X B), there was an interaction between circadian and group influences on CO (averaged
for baseline and tilt), i.e., presyncopal group generally had smaller CO and the group difference was much less at 300°-360° compared to other circadian phases (P = 0.037). We found no significant interactions between group and circadian influences on SV, EJT, and TPR.

At the end of head-up tilt. Before tilting down (10 seconds before putting the tilt table to the horizontal position), the non-presyncopal group showed no significant changes in SBP, DBP, SV, CO, and TPR, as compared to initial stage of tilt-up (Supplemental Figure XII). There was a slight increase in HR (P = 0.016), and a slight decrease in EJT (P = 0.031). In contrast, the presyncopal group showed overall (including trials with and without presyncope) significant decreases in SBP (P <0.001), DBP (P <0.001), SV (P <0.001), and CO (P = 0.0023) before tilting down, but no significant changes in HR, EJT, and TPR.

Within the presyncopal group, the decreases in SBP (P <0.001) and SV (P = 0.048) were more pronounced during the presyncope trials while the decreases in DBP (P <0.001) and CO (P <0.001) were only pronounced during the presyncope trials. EJT decreased only in trials without presyncope in the presyncopal group (P = 0.04). Though mean HR and TPR in all presyncope trials showed no significant changes before tilting down, we noted that the behaviors of these variables could be different in the presence of presyncope, depending on the timing of aborting the tests (see “Presyncope during tilt-table testing” in the Results section of the main manuscript). For instance, HR might still remain increased if subjects were tilted down early/immediately when signs/symptoms of presyncope just appeared; or HR could start to decrease dramatically if tilting down occurred when syncope was almost fully developed (Figure 2). Such a dynamic effect may explain the large variations of the HR and TPR changes before being tilted down in the 21 presyncope trials (Supplemental Figure XII).
Supplemental Tables

**Supplemental Table I.** Specification of the generalized linear mixed model (GLMM) for the assessment of the circadian distribution of presyncope events. Test outcomes of all 12 subjects (12x12=144 tests) were pooled together for the analysis. Results are presented in Figure 3B of the main manuscript.

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**Supplemental Table II.** Specification of the mixed models for the assessment of effects of tilt, group (presyncopal group: those with presyncope; non-presyncopal group: those who never experienced presyncope throughout the protocol), and their interactions on physiological variables. Standard least squares and restricted maximum likelihood methods were used for fitting mixed models. Data from the baseline and tilt conditions (in all 144 tests) were pooled together for the analysis. Results are presented in Figure 4 of the main manuscript.

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Supplemental Table III. Specification of the mixed models for the assessment of differences in baseline variables and their responses to tilt between trials with and without presyncope within the presyncopal group. Data of 6 subjects with presyncope experience (21 trials with presyncope and 51 without presyncope) during both the baseline and tilt conditions were pooled together for the analysis. Standard least squares and restricted maximum likelihood methods were used for fitting mixed models. Results are presented in Figure 5 of the main manuscript.

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Supplemental Table IV. Specification of the mixed models for the assessment of the effects of circadian phase and its interactions with tilt and group effects on physiological variables. $\theta$ is circadian phase determined from the core body temperature (see Supplemental Figures I-II). Data from both the baseline and tilt conditions from all 144 tests were pooled together for the analysis. Results are presented in Figure 6 and the Table of the main manuscript.

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Supplemental Figure I. Core body temperature of one subject throughout the 10-day forced desynchrony phase (twelve 20-h cycles) in the experimental protocol.
Supplemental Figure II. A schematic representation of circadian phase estimation and data binning. The phase, period ($\tau \approx 24$ h) and amplitude of circadian oscillations were estimated from least squares regression of core body temperature recording throughout the entire study. Circadian phase was assigned as $0^\circ$ at the time of CBT minimum ($t_{min}$) and each data point was assigned a circadian phase, i.e., for a data point at time $t < \tau$, the circadian phase $= 360^\circ (t - t_{min}) / \tau$. 
Supplemental Figure III. Responses of frequency-domain HRV indices to head-up tilt, and their differences between the non-presyncopal (black) and the presyncopal groups, and between completed trials (blue) and aborted trials (red) in the presyncopal group. Data are presented as mean±SE. Shown at the left corners of panels are P values for: (i) tilt effects (significant for all variables except for lnLF); (ii) mean group differences (P>0.1 for all variables); and (iii) the interaction between group and tilt stressor (only significant for TP). Also shown at the right corners of panels are P values for differences in (i) overall mean values (both baseline and tilt; P > 0.1 for all variables) and (ii) tilt responses (significant for lnLF and lnHF) between the 21 presyncpe cases and the other 51 trials without presyncpe within the presyncopal group.

Results were obtained from the mixed models specified in Supplemental Table II for group comparisons and in Supplemental Table III for within-group comparisons. “NS” indicates not significant (here, P always >0.1).
Supplemental Figure IV. Circadian influences on frequency-domain HRV indices and their responses to head-up tilt. The data (symbols) and the cosinor fits (lines) are plotted separately for baseline (black squares and continuous lines) and head-up tilt (circles and dashed lines). Gray bars indicate the average habitual sleep period when living outside of the laboratory. The data are presented as mean±SE across subjects. The results are double plotted to better visualize rhythmicity, with circadian phase on the lower abscissa and the corresponding habitual time of day on the upper abscissa. Shown are the mixed model derived P values for circadian influences (significant for TP, lnLF and lnHF) and interaction between tilt and circadian influences (not significant for all variables). There were no significant interactions between circadian and group effects for all variables. “NS” indicates not significant (here, P always >0.1). Results were obtained from the cosinor analyses using mixed-model ANOVAs (Supplemental Table IV).
Supplemental Figure V. Responses of low and high frequency powers of systolic blood pressure (SBP) fluctuations to head-up tilt, and their differences between the non-presyncopal (black) and the presyncopal groups, and between completed trials (blue) and aborted trials (red) in the presyncopal group. Log scales of low frequency (lnLF) and high frequency (lnHF) were used and data are presented as mean±SE. Shown at the left corners of panels are P values for: (i) tilt effects (significant for both lnLF and lnHF); (ii) mean group differences (P>0.1 for both variables); and (iii) the interaction between group and tilt stressor (P > 0.1 for both variables). Within the presyncopal group, lnHF and lnLF were not significantly different between the 21 presyncope cases and the other 51 trials without presyncope. “NS” indicates not significant (here, P always >0.1). Results were obtained from the mixed models specified in Supplemental Table II for group comparisons and in Supplemental Table III for within-group comparisons.
Supplemental Figure VI. Log scales of low and high frequency powers of systolic blood pressure at different circadian phases. The data (symbols) and the cosinor fits (lines) are plotted separately for baseline (black squares and continuous lines) and head-up tilt (circles and dashed lines). Gray bars indicate the average habitual sleep period when living outside of the laboratory. The data are presented as mean±SE across subjects. The results are double plotted to better visualize rhythmicity, with circadian phase on the lower abscissa and the corresponding habitual time of day on the upper abscissa. Shown are the mixed model derived P values for circadian influences (not significant for both lnLF and lnHF) and interaction between tilt and circadian influences (significant for lnLF). There were no significant interactions between circadian and group effects on both variables. Results were obtained from the cosinor analyses using mixed-model ANOVAs (Supplemental Table IV).
Supplemental Figure VII. Distribution of presyncope events across 12 sequential 20-h cycles throughout the forced desynchrony protocol. There were 11 presyncope events during the first 6 cycles and 10 during the last 6 cycles, thus there was no simple systematic effect of time into the protocol on incidence of presyncope, except for a clear underlying endogenous circadian rhythm indicated by two clear peaks in the frequency of presyncope events at Cycle 3 and Cycle 9 when the head-up tilt tests were performed during the biological night (the corresponding circadian phase bin of 180°). The upper abscissa of the figure is the average time of day (for the 6 presyncopal subjects) when head-up tilt tests were performed.
Supplemental Figure VIII. Distribution of tilt duration in 21 head-up tilt tests with presyncope.

Most of presyncope events occur between 8-12 minutes after being tilted up.
Supplemental Figure IX. Average probability of presyncope occurrence across all circadian phases. Gray bars indicate the average habitual sleep period when living outside of the laboratory. Error bars are standard errors. Results were double plotted to better visualize rhythmicity, with circadian phase on the lower abscissa and the corresponding habitual time of day on the upper abscissa. A generalized linear mixed model revealed significant level of circadian influence when including all 21 presyncope cases ($P = 0.028$). A similar circadian variation was observed ($P = 0.042$) when excluding 4 presyncope cases when tests were aborted mainly due to symptoms without significant hypotension (assigning the 4 cases as completed trials).
**Supplemental Figure X.** Responses of stroke volume (SV), cardiac output (CO), ejection time (EJT) and total peripheral resistance (TPR) to head-up tilt, and their differences between the non-presyncopal (black) and the presyncopal groups, and between completed trials (blue) and aborted trials (red) in the presyncopal group. Results were obtained from finger plethysmography and data are presented as mean±SE. Shown at the left corners of panels are P values for: (i) tilt effects (significant for all variables); (ii) mean group differences (P>0.1 for all variables); and (iii) the interaction between group and tilt stressor (significant for TPR). Within the presyncopal group, all these four variables and their responses to tilt were not significantly different between the 21 presyncope cases and the other 51 trials without presyncope (as shown by “NS” shown at right corners). Results were obtained from the mixed models specified in **Supplemental Table II** for group comparisons and in **Supplemental Table III** for within-group comparisons. “NS” indicates not significant (here, P always >0.1).
Supplemental Figure XI. Circadian influences on stroke volume (SV), cardiac output (CO), ejection time (EJT) and total peripheral resistance (TPR), and their responses to head-up tilt. The data (symbols) and the cosinor fits (lines) are plotted separately for baseline (black squares and continuous lines) and head-up tilt (circles and dashed lines). Gray bars indicate the average habitual sleep period when living outside of the laboratory. The data are presented as mean±SE across subjects. The results are double plotted to better visualize rhythmicity, with circadian phase on the lower abscissa and the corresponding habitual time of day on the upper abscissa. Shown are the mixed model derived P values for circadian influences (significant for CO and EJT). There was no significant interaction between tilt stressor and circadian phase for any of these variables. Results were obtained from the cosinor analyses using mixed-model ANOVAs (Supplemental Table IV).
Supplemental Figure XII. Changes in physiological variables at the end of head-up tilt as compared to at the initial stable phase of tilt. Shown are the changes between 10 seconds before being tilted down and ~1 minute after being tilted up in systolic blood pressure (ΔSBP), diastolic blood pressure (ΔDBP), heart rate (ΔHR), stroke volume (ΔSV), cardiac output (ΔCO), ejection time (ΔEJT) and total peripheral resistance (ΔTPR). Significant changes are indicated by *(P < 0.05), **(P < 0.001), and ***(P<0.0001). Results were obtained from the mixed model ANOVAs in which subject was included as a random effect for intercept.
Figure XIII. Endogenous circadian rhythms in subjective scores of A. “nausea”, B. “general discomfort”, and C. “feeling hot” during tilt-testing. The binned data (symbols) and the cosinor model fits (lines) were plotted separately for the non-presyncopal group (squares and lines) and the presyncopal group (circles and lines). Gray bars indicate the average habitual sleep period when living outside of the laboratory. Data are presented as mean±SE across subjects. The results are double plotted to better visualize rhythmicity, with circadian phase on the lower abscissa and the corresponding habitual time of day on the upper abscissa. Shown are the mixed model derived P values for: (i) group differences (not significant or “NS” for all variables); (ii) circadian influences (significant for all variables); and (iii) interaction between group and circadian phase (significant for the nausea score). Results were obtained from the cosinor analyses using mixed-model ANOVAs in which effects of group, circadian and their interaction were included as fixed effects and subject was as a random factor for intercept.
Supplemental References


