Time of day affects the frequency and duration of breathing events and the critical closing pressure during NREM sleep in participants with sleep apnea

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1John D. Dingell Veterans Affairs Medical Center, Detroit, Michigan; 2Department of Physiology, Wayne State University School of Medicine, Detroit, Michigan; 3Department of Internal Medicine, Wayne State University School of Medicine, Detroit, Michigan; 4Department of Otolaryngology-Head and Neck Surgery, Wayne State University School of Medicine and Karmanos Cancer Institute, Detroit, Michigan; and 5Department of Biomedical Engineering, Wayne State University Detroit, Michigan

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El-Chami M, Shaheen D, Ivers B, Syed Z, Badr MS, Lin HS, Mateika JH. Time of day affects the frequency and duration of breathing events and the critical closing pressure during NREM sleep in participants with sleep apnea. J Appl Physiol 119: 617–626, 2015. First published July 16, 2015; doi:10.1152/japplphysiol.00346.2015.—We investigated if the number and duration of breathing events coupled to upper airway collapsibility were affected by the time of day. Male participants with obstructive sleep apnea completed a constant routine protocol that consisted of sleep sessions in the evening (10 PM to 1 AM), morning (6 AM to 9 AM), and afternoon (2 PM to 5 PM). On one occasion the number and duration of breathing events was ascertained for each sleep session. On a second occasion the critical closing pressure that demarcated upper airway collapsibility was determined. The duration of breathing events was consistently greater in the morning compared with the evening and afternoon during N1 and N2, while an increase in event frequency was evident during N1. The critical closing pressure was increased in the morning (2.68 ± 0.98 cmH2O) compared with the evening (1.29 ± 0.91 cmH2O; P < 0.02) and afternoon (1.25 ± 0.79; P ≤ 0.01). The increase in the critical closing pressure was correlated to the decrease in the baseline partial pressure of carbon dioxide in the morning compared with the afternoon and evening (r = −0.73, P ≤ 0.005). Our findings indicate that time of day affects the duration and frequency of events, coupled with alterations in upper airway collapsibility. We propose that increases in airway collapsibility in the morning may be linked to an endogenous modulation of baseline carbon dioxide levels and chemoreflex sensitivity (12), which are independent of the consequences of sleep apnea.

circadian rhythm; breathing event duration and frequency; passive and active critical closing pressure; upper airway muscle activity

CLINICAL STUDIES HAVE REPORTED that independent of sleep stage and body position, the number (13, 48) and duration (4, 5, 20, 36, 48) of breathing events increase throughout the night during non-rapid-eye-movement sleep, although to our knowledge no studies have examined if these differences manifest in a similar manner in stages N1 and N2 of non-rapid-eye-movement sleep. Likewise, computer simulations have suggested that a reduction in the apnea-hypopnea index during daytime compared with nighttime sleep may occur (49). This latter postulation has been reported in a small number of hypertensive men, although other confounding factors may have influenced the findings (45).

The potential mechanisms underlying the increase in the number and duration of breathing events across a 24-h period are likely multifactorial and phenotypically dependent. One possibility is that increases in chemoreflex sensitivity, coupled with a reduction in the carbon dioxide reserve, contributes to the progressive increase in breathing events across the night (12, 25, 26, 28–30). This possibility is well founded in that enhanced chemoreflex sensitivity promotes the occurrence of both central and obstructive breathing events (8–10, 56). The results from many studies indicate that the increase in chemoreflex sensitivity across the night could be the result of exposure to intermittent hypoxia, a hallmark of sleep apnea (6, 14, 15, 19, 27, 53). However, we recently showed that increases in chemoreflex sensitivity and decreases in the carbon dioxide reserve were evident during sleep in the morning compared with the evening and afternoon; independent of physiological hallmarks typically associated with sleep apnea (i.e., intermittent hypoxia and sleep fragmentation) (12).

Previous studies have also addressed if increases in the arousal threshold or effective recruitment threshold of upper airway muscles are responsible for reported increases in apnea duration from the beginning to the end of the night. Based on a series of studies, a number of investigators (2, 4, 36) proposed that inflammation, edema, neural damage in response to snoring related vibrations, and forceful collapse of the larynx progressively dampen excitatory inputs from upper airway sensory receptors from the beginning to the end of the night, leading to alterations in the arousal and/or effective recruitment threshold. Thus the effect of time of day on the arousal and/or effective recruitment threshold was linked to factors that are a consequence of apnea and not endogenously modulated by a circadian rhythm. Traumatization of the upper airway could also account for increases in the frequency of apnea events since collapsibility of the upper airway could be impacted by upper airway muscle fatigue associated with exposure to intermittent hypoxia (3, 34, 42) and dampening of excitatory sensory inputs from the upper airway receptors (4).

Despite these findings, we were interested in determining if alterations in upper airway collapsibility occur in accordance with the time of day, independent of deleterious factors typically associated with sleep apnea. To our knowledge no studies have directly examined the effect of time of day on upper airway muscle collapsibility in humans with sleep apnea. Nevertheless, existing evidence indicates that upper airway collapsibility, via modulation of neuromuscular properties, could be affected by the time of day. Many studies in humans

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(see Ref. 57 for review) have shown that force, along with other measures of muscle function [i.e., rate of tension development and one-half relaxation time (24)], in skeletal muscle ranging from the abductor pollicis (24) to leg extensor muscles (47), is enhanced in the late afternoon and evening compared with the early morning. In addition, studies in other animals have reported that the concentration of a neuromodulator involved in the control of upper airway muscle activity (i.e., serotonin) along with receptor subtypes (i.e., 5HT2A) is modulated by the time of day (1, 31, 50, 52).

Therefore, the primary aims of the present investigation were twofold. First, we examined the effect of time of day on the duration and frequency of breathing events during non-rapid-eye-movement sleep in the evening, morning, and afternoon in individuals with sleep apnea using a constant routine protocol. This aim was completed to confirm the results from previous clinical studies and to add to these findings by exploring the characteristics of breathing events during N1 and N2 sleep at three time periods throughout the 24-h cycle. We hypothesized that the duration and frequency of breathing events would be greater during sleep in the morning compared with the evening and afternoon. The second aim of the study was to examine if collapsibility of the upper airway, via measures of the critical closing pressure, was altered when these measures were compared during non-rapid-eye-movement sleep in the evening, morning, and afternoon. We hypothesized that the critical closing pressure would be more positive in the morning compared with the evening and afternoon if the modulation of upper airway muscle function is similar to that reported previously for other skeletal muscles.

METHODS

Protocol. The Human Investigation Committees of Wayne State University School of Medicine and John D. Dingell Veterans Affairs Medical Center approved the experimental protocol. Thirteen male participants with untreated pure or predominantly obstructive sleep apnea but no other comorbidities (e.g., heart and lung disease, hypertension, and obesity), completed the protocol. All the participants who completed the protocol visited the laboratory on six occasions (Fig. 1). During the first visit to the laboratory, written informed consent was obtained and thereafter a physical examination, health and lifestyle questionnaires, blood pressure, lung volume measures, and a 12-lead ECG were completed. After ensuring that the inclusion criteria were met, participants completed a baseline nocturnal polysomnogram to confirm the presence of obstructive sleep apnea (visit 2). Upon verification, participants were enrolled in the protocol and their sleep was monitored at home for 2 wk, using an actigraph watch (Activwatch Spectrum; Philips Respironics, Murraysville, PA) before the planned physiological measurements were obtained on visits 4–6 (see subsequent paragraph for further details). During the 2-wk time period we requested that the participants adhere to a regular sleep-wake schedule with a sleep onset time between 10 and 11 PM and a wake time of 7–8 AM. We also requested that the participants avoid daytime napping. During this time period the participants returned to the laboratory (i.e., visit 3) to determine the therapeutic continuous positive airway pressure required to maintain airway patency. In addition, a "practice trial" using the methodology and procedures required to determine the critical closing pressure was completed so that each participant was accustomed to the procedures.

After the 2-wk time period, participants returned to the laboratory on three separate occasions (i.e., visits 4–6). Each visit was separated by a minimum of 7 days. Participants maintained a regular sleep-wake schedule, which was monitored with the actigraph watch, and were not treated with continuous positive airway pressure during the 7-day interval that separated each visit. Participants were asked to abstain from alcohol and caffeinated beverages at least 1 day before the visit. On the day of each study, participants arrived at the laboratory at ~8:00 PM. Upon arrival, the participants ingested a radiotelemetry pellet (CorTemp Sensor, Palmetto, FL), which measured core body temperature every 10 s throughout each visit. This measure was used to establish the nadir of core body temperature. Following instrumentation, the participants completed a constant routine protocol. The protocol was comprised of sleep sessions in the evening, morning and afternoon that were 3 h in duration (i.e., 10 PM–1 AM, 6–9 AM, and 2–5 PM). Subsequent to the evening and morning sleep sessions, participants were placed in a semirecumbent position during wakefulness. At the onset of each wake session, participants watched a movie for ~120 min and immediately thereafter read for ~90 min. Ninety minutes before the morning or afternoon sleep session, the participants sat quietly and did not engage in any activity. During each wake session, participants received small snacks every 95 min composed of ~15% fat, 75% carbohydrate, and 10% protein. Moreover, participants received up to a maximum of 1 liter of water over the length of the constant routine protocol. During wakefulness, the participants were in a dimly lit (i.e., 30 lux) laboratory that was randomized.

![Fig. 1. A schematic diagram showing the protocol completed by the participants. *8 of 13 participants completed visit 7.](http://jap.physiology.org/Downloadedfrom)
was 1.7 measured during each sleep session (Fig. 2). During each session, (i.e., the pressure associated with collapse of the upper airway) was subsequently (12). Subsequent to this publication, 3 additional participants were enrolled, so that measures of the critical closing pressure were obtained from 13 participants.

To assess upper airway collapsibility, the critical closing pressure (i.e., the pressure associated with collapse of the upper airway) was measured during each sleep session (Fig. 2). During each session, airway patency was initially maintained using a holding pressure that was 1.7 ± 0.4 cmH2O less than the therapeutic pressure determined during visit 3. The holding pressure generated a reduction in airflow of 11.5 ± 3.1% and was employed to prevent overdistention of the airway. Measurement of the critical closing pressure was performed by reducing the mask pressure in a stepwise fashion by increments of 1–2 cmH2O (Pcrit 3000, version 1.0; Philips Respironics, Murrysville, PA) for a duration of three to five breaths. Each step down in pressure was separated by a 1-min recovery period at the holding pressure. Stepwise reductions in pressure continued until the airway was fully collapsed, which was defined by measures of airflow <10% percent of baseline.

We were also interested in determining if measures of the critical closing pressure were similar at a given time of day using a well-established method (32) characterized by consecutive 1–2 cmH2O reductions in mask pressure, which were sustained for a duration of 5 min and were not separated by a recovery period at the holding pressure. We rationalized that establishing a holding pressure before a step-down in mask pressure lasting three to five breaths would be accompanied by upper airway muscle activity. Moreover, we considered that reductions in airway pressures are detected in a short period of time and result in the activation of the upper airway muscles within 50 ms (16, 17). Consequently, we hypothesized that independent of the length of time maintained after a step down in pressure the critical closing pressure that demarcated collapse of the airway would be similar. If supported, our findings would provide a rationale for using the method with step downs in pressure of short duration, because the overall time required to implement the methodology would be shorter and decrease the probability of disrupting sleep architecture. To compare the two methods used to measure the critical closing pressure, 8 of the 13 participants returned to the laboratory for an additional visit (Fig. 1, visit 7). During this visit we measured the critical closing pressure during sleep in the evening, morning and afternoon using the method characterized by step downs in mask pressure, which were sustained for 5 min (32).

**Instrumentation.** During the sleep studies the monitoring montage included an electroencephalogram (C3/A2, C4/A1, O1/A2, and O2/A1), electrooculograms, submental electromyogram, and an electrocardiogram. Chest wall and abdominal movements were measured using inductive plethysmography (Respirtrace; Ambulatory Monitoring, Ardsley, NY). Airflow and breath timing (inspiratory and expiratory time) were measured using a pneumotachometer (model RS100-HR; Hans Rudolph, Shawnee, KS) attached to a nasal mask. Oxygen saturation (arterial O2 saturation) was measured with a pulse oximeter (Biox 3700; Ohmeda, Boulder, CO). Measures of end-tidal oxygen (model 17515; Vacumed, Ventura, CA) and end-tidal carbon dioxide (model 17518; Vacumed) were obtained from air expired into sampling tubes attached to ports on the nasal mask. Mask pressure was measured using a pressure transducer attached via tubing to a port on the nasal mask. Upper airway pressure was measured using a transducer tipped catheter (Mikro-Cath 825-0101; Millar, Houston, TX) to confirm apnea and ascertain the presence of flow limitation. All physiological variables were analog to digitally converted at a sampling frequency of 100 Hz/channel and input into a computer using a commercially available software package (gamma version 4.0; Astro-Med, West Warwick, RI). The cardiorespiratory variables were also input into a second computer using a commercially available software package (WinDaq; Dataq Instruments, Akron, OH).

**Data analysis.** All polygraphic sleep studies were analyzed for sleep stage, arousals, and respiratory-related events according to standard published criteria (18). An event characterized by the absence of inspiratory airflow for a minimum of 10 s was identified as an apnea. A hypopnea was defined as an event accompanied by a >50% reduction in airflow that lasted for a minimum of 10 s and was also accompanied by either an arousal or a ≥3% reduction in oxygen saturation in the absence of an arousal. During the fourth visit, the number and duration of apneas and hypopneas, as well as the decrease in oxygen saturation during each event, were determined for N1 and N2 of non-rapid-eye-movement sleep for each sleep session. Analyses of events recorded in N3 were not included in the results because the overall time spent in N3 was not sufficient to make adequate comparisons between sleep sessions within or across participants (see Table 2).

For each participant, baseline measures of minute ventilation and the partial pressure of end-tidal carbon dioxide were obtained initially from a 2-min period of stable non-rapid-eye-movement sleep before completing the protocol on visit 5 or 6. Baseline measures for 10 of the 13 participants were published previously (12). To obtain measures of the critical closing pressure in 13 participants, baseline values of airflow and mask pressure were determined initially using the last 5 breaths measured at the holding pressure before the initial step down (Fig. 2). Thereafter, with every reduction in pressure the resultant peak flow was determined from the second or third breath. The breath selected displayed the greatest limitation in airflow (Fig. 2). To obtain the critical closing pressure using the method that incorporated pressure step-downs of longer duration (n = 8 participants), breath-by-
breath measures of airflow recorded from the last 2 min of the baseline holding period were analyzed. Thereafter, flow was measured from breaths recorded during the final 2 min of each pressure step down to determine the critical closing pressure. If participants aroused from sleep before determining the critical closing pressure during this visit, this measure was extrapolated using a second-order polynomial regression from the available pressure steps recorded before arousal. All measures of the critical closing pressure were obtained during stable N2 of non-rapid-eye-movement sleep.

We were interested in determining the correlation between the critical closing pressure and baseline measures of the apnea/hypopnea index. To examine this relationship we averaged the critical closing pressures measured in the evening, morning and afternoon. In addition, to determine the correlation between the partial pressure of end-tidal carbon dioxide and the critical closing pressure we averaged values of end-tidal carbon dioxide and averaged the critical closing pressure, measured in the evening, morning, and afternoon. To further explore this relationship, we also averaged end-tidal carbon dioxide and critical closing pressure values in the evening and afternoon, since measures of the critical closing pressure were similar for these two sessions. Subsequently, the change in end-tidal carbon dioxide and critical closing pressure in the morning compared with the average of the evening/afternoon was determined to correlate these measures.

Measurements of core body temperature were purified to eliminate small fluctuations in temperature associated with movements during wakefulness that occurred during feeding or bathroom breaks. Thereafter, the temperature was fit with a cosine wave \( y = m + a \cos (2\pi t / o) + \phi \), where \( y \) is the temperature, \( m \) is the circadian rhythm adjusted mean (i.e., mesor), \( a \) is the amplitude of the circadian rhythm, \( t \) is time (hours), \( o \) is the period of the circadian rhythm, and \( \phi \) is the phase angle. Once the phase and period of the core body temperature were established for an individual, this information was used to assign a circadian phase (from 0 to 359°) to each minute, with 0° corresponding to the minimum of the waveform fitted to the core body temperature data. The plotted data were then used to determine the session that corresponded to the core body temperature nadir.

**Statistical analysis.** A one-way repeated-measures analysis of variance in conjunction with Fisher’s least square difference post hoc test was used to compare sleep efficiency measures in the evening, morning and afternoon. A similar analysis was used to compare the measures of the critical closing pressure (\( n = 13 \)) and baseline measures of carbon dioxide in the evening, morning and afternoon. A Kruskal-Wallis one-way ANOVA on ranks combined with Student-Newman-Keuls post hoc test was used to compare the partial pressure of end-tidal carbon dioxide in the evening, morning and afternoon. A two-way repeated-measures ANOVA in conjunction with Fisher’s least square difference post hoc test was used to compare the percentage of time spent in N1 and N2 of non-rapid-eye-movement sleep during the evening, morning, and afternoon sleep sessions. The two factors in the analysis were sleep stage (i.e., N1 and N2) and time of day (i.e., evening, morning and afternoon). A similar analysis was used to compare measures of breathing event duration, apnea/hypopnea indexes, decreases in oxygen saturation, and the critical closing pressure measured using two separate methods. For each variable, the factors used in the analysis were sleep stage and time of day, with the exception of the statistical analysis of the critical closing pressure. In this case, the factors were time of day and method (i.e., brief or prolonged step-downs in pressure) used to determine the critical closing pressure. A Pearson correlation analysis was used to determine if 1) the critical closing pressure was correlated to the baseline apnea/hypopnea index; 2) the average baseline measures of end-tidal carbon dioxide were correlated to the average critical closing pressure measured during the evening, morning and afternoon; and 3) the change in the partial pressure of end-tidal carbon dioxide was correlated to the change in the critical closing pressure from the morning to the evening and afternoon. Data are presented as means \( \pm \) SE. \( P \leq 0.05 \) was considered statistically significant.

**RESULTS**

Table 1 shows the anthropometric variables obtained for the group. Collectively, the participants were young to middle age, and not obese, as indicated by the body mass index. The apnea/hypopnea index determined from the screening sleep study (i.e., visit 2) ranged from mild to severe according to standard criteria. The level of oxygen desaturation achieved during apneic/hypopneic events was mild even in those participants considered to have severe sleep apnea. Systolic and diastolic blood pressure measurements were within normal limits and the Epworth sleepiness scale indicated a history of mild sleepiness (Table 1). The average therapeutic pressure required to eliminate apnea during sleep on visit 3 in the obstructive sleep apnea participants was 11.2 \( \pm \) 0.8 cmH2O. Average measures of core body temperature, recorded during visit 4 of the protocol, are shown in Fig. 3. The nadir of temperature was evident in the early morning during visit 4 (Fig. 3) and in the course of the visit used to measure the critical closing pressure.

During visit 4, the participants spent 73.4 \( \pm \) 4.9, 78.0 \( \pm \) 2.7, and 71.4 \( \pm \) 3.6%, of the total session time (i.e., 3 h) in N1 and

**Table 1. Baseline anthropometric, blood pressure, and sleep measurements**

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>29.5 ± 1.9</td>
</tr>
<tr>
<td>Height, cm</td>
<td>174.5 ± 4.0</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>82.6 ± 2.7</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.4 ± 0.6</td>
</tr>
<tr>
<td>Systolic pressure, mmHg</td>
<td>119.2 ± 2.6</td>
</tr>
<tr>
<td>Diastolic pressure, mmHg</td>
<td>72.7 ± 2.8</td>
</tr>
<tr>
<td>Apnea/hypopnea index, events/h</td>
<td>43.5 ± 4.4</td>
</tr>
<tr>
<td>Lowest oxygen desaturation during</td>
<td>87.2 ± 1.2</td>
</tr>
<tr>
<td>apnea, %</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td>7 African Americans, 4 Caucasian, 1 Asian, 1 Indian</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE.
N2 of non-rapid-eye-movement sleep in the evening, morning and afternoon, respectively (P ≥ 0.47). The percentage of time spent in N1 or N2 for a given 3-h sleep session was not significantly different between the evening, morning and afternoon (Table 2; P ≥ 0.85). Figure 4A shows the average duration of breathing events during N1 and N2 of non-rapid-eye-movement sleep during the sleep sessions completed on visit 4. The duration of breathing events was greater in the morning compared with the afternoon and evening in N1 and N2 of non-rapid-eye-movement sleep (P ≤ 0.001). In addition, the duration of breathing events was greater during N2 compared with N1 in the evening, morning, and afternoon (P ≤ 0.001).

The apnea/hypopnea index was greater in the morning compared with the evening (P ≤ 0.01) and afternoon (P ≤ 0.02) in N1 of non-rapid-eye-movement sleep (Fig. 4B). In contrast, the apnea/hypopnea index during the evening, morning, and afternoon was similar across the three sleep sessions in N2 (Fig. 4B). The apnea/hypopnea index measured during N2 was greater compared with N1 during the evening (P ≤ 0.02) and afternoon sleep sessions (P ≤ 0.04). This difference was not evident throughout the morning sleep session. Baseline oxygen saturation measures were similar across sleep sessions in N1 (evening: 98 ± 0.3%; morning: 99 ± 0.2%; and afternoon: 99 ± 0.2%) and N2 (evening: 98 ± 0.3%; morning: 98 ± 0.2%; and afternoon: 98 ± 0.3%). In addition, the decrease in oxygen saturation during breathing events was similar in the morning compared with the afternoon and evening in N1 and N2 (Fig. 4C). The decrease in oxygen saturation during breathing events in N2 was significantly greater compared with N1 independent of the time of day (P ≤ 0.002).

The critical closing pressure using brief step-downs in pressure (i.e., 3–5 breaths) was more positive in the morning compared with the evening (P ≤ 0.02) and afternoon (P ≤ 0.01; n = 13; Fig. 5A). These relationships were unchanged (P ≤ 0.001) when the critical closing pressure was determined using pressure step-downs of longer duration (n = 8; Fig. 5B, right). No difference in the critical closing pressure was evident in the evening, morning, or afternoon when measures obtained from eight participants using pressure step-downs of short (3 breaths) and long (5 min) duration were compared (Fig. 5B). The average critical closing pressure was correlated to the baseline apnea/hypopnea index (r = 0.60, P ≤ 0.03; r = 0.74, P ≤ 0.01 with one outlier removed; see Fig. 6A for additional details).

As previously reported for 10 of the 13 participants, baseline measures of the partial pressure of end-tidal carbon dioxide were lowest in the morning compared with the evening and afternoon (12). This relationship remained unchanged with data from three additional participants included (evening 41.4 ± 0.6 vs. morning 40.2 ± 0.4 vs. afternoon 40.6 ± 0.5 mmHg; P ≤ 0.01). Interestingly, average baseline measures of the partial pressure of end-tidal carbon dioxide were correlated to the average of the critical closing pressure obtained from the three sleep sessions (r = −0.59, P ≤ 0.04). The highest baseline levels of PETCO2 were coupled to a less positive or negative critical closing pressure and vice versa. In addition, the change in carbon dioxide in the morning, relative to the average value measured in the evening and afternoon, was strongly correlated to the change in critical closing pressure in the morning (r = −0.73, P ≤ 0.005; Fig. 6B). The greater the decrease in baseline partial pressure of carbon dioxide the

Table 2. Time spent in a given stage of sleep as a percentage of session time

<table>
<thead>
<tr>
<th>Session</th>
<th>Evening</th>
<th>Morning</th>
<th>Afternoon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wake, %</td>
<td>24.7 ± 5.1</td>
<td>9.3 ± 2.3</td>
<td>19.9 ± 3.8</td>
</tr>
<tr>
<td>N1, %</td>
<td>22.7 ± 3.7</td>
<td>19.2 ± 2.8</td>
<td>20.3 ± 2.5</td>
</tr>
<tr>
<td>N2, %</td>
<td>47.6 ± 3.4</td>
<td>53.0 ± 4.0</td>
<td>49.0 ± 3.7</td>
</tr>
<tr>
<td>N3, %</td>
<td>3.1 ± 1.6</td>
<td>5.8 ± 2.5</td>
<td>2.1 ± 1.1</td>
</tr>
<tr>
<td>REM, %</td>
<td>1.9 ± 0.9</td>
<td>12.6 ± 2.9</td>
<td>8.7 ± 2.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. REM, rapid-eye-movement sleep. N1–N3, stages of REM.

Fig. 4. Histograms which show the mean values for the duration of breathing events (A), the apnea hypopnea index (B), and the decrease in oxygen saturation (C), for N1 and N2 of non-rapid-eye-movement sleep in the evening, morning, and afternoon sleep sessions. Note that breathing event frequency was greater in the morning compared with the afternoon and evening in N1 and the duration of breathing events was greater in the morning in both N1 and N2. Also note that the decrease in oxygen saturation was similar and independent of breathing event duration. *Significantly different from the evening and afternoon. ‡Significantly different from N1.
DISCUSSION

In the present investigation we employed a constant routine protocol to measure the characteristics of breathing events on one occasion, and the critical closing pressure on another, during non-rapid-eye-movement sleep in the evening, morning, and afternoon in participants with sleep apnea. Our primary findings revealed that the duration of breathing events increased during N1 and N2 of non-rapid-eye-movement sleep in the morning compared with the evening and afternoon. Additionally, an increase in the frequency of breathing events was evident during N1 in the morning. Our results also showed that the critical closing pressure of the upper airway was more positive in the morning compared with the evening and afternoon. Secondary, we also showed that the duration and frequency of breathing events were greater in N2 compared with N1 and that measures of the critical closing pressure were independent of the duration of the reduction in pressure used to obtain these measures.

Methodology. Our participants did not suffer from other comorbid conditions (i.e., diabetes, cardiovascular disease, and obesity). Thus the potential influence of these comorbidities on the critical closing pressure was controlled. On the other hand, our results may not be representative of the responses of older, obese patients who suffer from comorbid conditions. Accordingly, the effect of time of day on the critical closing pressure could vary and be dependent on age and the presence of comorbidities. In addition, our study was limited to investigating the effect of time of day on the critical closing pressure in men. Inclusion in the study was not limited to this sex; rather it was by happenstance that the participants available to complete the protocol, which was extensive and required a number of visits to the laboratory, were male. Consequently, future studies are necessary to determine whether the effect of time of day on chemoreflex properties is sex dependent.

The critical closing pressure is determined by both neuromuscular and nonneuromuscular properties. Nonneuromuscular properties and neuromuscular properties that influence upper airway collapsibility may be separated if the airway is studied under conditions of hypo and hypertonia, respectively.

Fig. 5. Histograms that show the mean critical closing pressure in the evening, morning and afternoon measured using brief step-downs in pressure for 3–5 breaths (n = 13; A) and step-downs in pressure lasting 3–5 breaths and 5 min (n = 8; B). Note that independent of the duration of each step-down in pressure, the critical closing pressure that demarcated the upper airway collapsibility was greater in the morning compared with the evening and afternoon. PCrit, critical closing pressure. *Significantly different from the evening and afternoon.

Fig. 6. A: scatterplot that shows the relationship between the average critical closing pressure, measured in the morning, evening, and afternoon for each participant, and the baseline apnea/hypopnea index. Note that the open circle represents a value that was significantly outside of the 99% confidence intervals shown on the plot. B: scatterplot that shows the correlation between the change in carbon dioxide in the morning compared with measures in the evening and afternoon, and the change in the critical closing pressure in the morning compared with values measured in the evening and afternoon.
(32, 46). However, we chose not to perform our measures under separate conditions because we were concerned that measures of the critical closing pressure under both conditions would not be possible in some participants given the duration of our sleep sessions. Thus, to ensure that the upper airway muscles were active, before completing brief step-downs in pressure, we maintained a holding pressure below the therapeutic pressure. This reduction in pressure was accompanied by a flow reduction. Thereafter, a negative pressure, relative to the holding pressure, was rapidly applied. Based on published studies we speculate that each brief step-down in pressure activated the negative pressure reflex within a short time period [i.e., 50 ms (16, 17)] resulting in a neuromuscular response that was affected by the time of day. To support our contention we subsequently measured the critical closing pressure using step-downs of longer duration, during which time neuromuscular responses were undoubtedly influenced by the interaction between mechanoreceptors and chemoreceptors (32). We surmised that if the critical closing pressure using the brief step-downs in pressure was principally a reflection of nonneuromuscular influences on the upper airway, contrary to our postulation, then the critical closing measurements would be more positive compared with the critical closing pressure measurements attained using the prolonged step-downs in pressure. This was not the case, since measures of the critical closing pressure were similar and independent of the method used. Our findings suggest that the presentation of additional stimuli that accompany prolonged step-downs in pressure might interact in a complex fashion to produce a critical closing pressure similar to that induced by a brief negative pressure pulse. Alternatively, the similar measures could indicate a reduced upper airway neuromuscular responsiveness to added stimuli that accompany prolonged step-downs in pressure in individuals with sleep apnea. This possibility has been reported previously (33).

Arousal state effects on breathing event characteristics. A secondary finding from our investigation was that the frequency and duration of breathing events were greater during N2 compared with N1. These results are contrary to the findings of Ratnavadivel et al. (41), which showed that the frequency of breathing events was reduced in N2 compared with N1. Ratnavadivel et al. (41) did not report the duration of breathing events and the corresponding decrease in oxygen saturation. It is possible that breathing event duration increased accompanied by greater reductions in oxygen saturation in N2 compared with N1, which is more in line with our results. On the other hand, the discrepancy between our results and previous findings could be a reflection of the quality of N2 sleep and the level of arousal following reestablishment of airway patency. Transitions from N1 to N2 sleep in individuals with sleep apnea are typically characterized by continued promotion of apneic events, while the transition from N2 to N3 is often linked to improved upper airway stability and decreases in event frequency (11). Although direct comparisons of sleep depth in N2 between studies is not possible, it is interesting to note that participants spent ~22% of non-rapid-eye-movement sleep in slow wave sleep in the investigation of Ratnavadivel et al. (41), while participants in our study spent little time in N3 (see Table 2).

Time of day effects on breathing event characteristics and upper airway collapsibility. Published findings have reported that the frequency and/or duration of breathing events increases during the second compared with the first half of the night, independent of sleep stage (4, 5, 13, 20, 36, 48). We have extended these findings by showing that the increase in the frequency and duration of breathing events during sleep in the morning are not only elevated compared with measures obtained during sleep in the evening but also compared with sleep in the afternoon. In previous studies, measures obtained during non-rapid-eye-movement sleep were separated from rapid eye movement sleep to explore the time of day effect on event frequency and duration independent of sleep stage (4, 5, 20, 36, 48). Although the potential confounding influence of rapid eye movement sleep was controlled, the various stages of non-rapid-eye-movement sleep were presumably combined in some investigations, since controls for non-rapid-eye-movement sleep stage were undefined (5, 13, 48). Consequently, increases in event frequency or duration during the second compared with the initial half of the night could have been a reflection of alterations in the contribution of N1, N2 and N3 to non-rapid-eye-movement sleep. Other studies limited analyses to N2 of non-rapid-eye-movement sleep (4, 20, 36). Thus time of day effects on breathing event characteristics could potentially be specific to N2 of non-rapid-eye-movement sleep. In the present investigation we explored if the effect of time of day on frequency and duration of events differs between N1 and N2. Our results showed that the frequency of events increased in the morning compared with the evening and afternoon in N1 but not in N2. Alternatively, the duration of breathing events increased in the morning compared with the evening and afternoon independent of the stage of non-rapid-eye-movement sleep.

If all conditions, with the exception of event duration, remained constant for a given sleep session, one would anticipate that the decrease in oxygen saturation would be greater during prolonged events. This was not the case, since the decrease in oxygen saturation was similar across sleep sessions within N1 and N2. Consequently, a longer time interval was required to attain a similar decrease in oxygen saturation. It is likely that the degree of oxygen desaturation was similar across sleep sessions because of differences in metabolic rate during sleep in the morning compared with the afternoon and evening. Indeed, we recently reported that baseline measures of minute ventilation, the partial pressure of end-tidal carbon dioxide, and core body temperature measured from 10 of the 13 participants in the present investigation were significantly less in the morning compared with the evening and afternoon (12). The addition of three participants did not alter this finding.

In support of the effect that time of day has on breathing event frequency and duration, we also showed that the critical closing pressure that demarcates upper airway collapsibility was greater in the morning compared with the evening and afternoon. Our study did not directly address if an inherent modulation of tissue properties or upper airway neuromuscular function was responsible for the increase in critical closing pressure in the morning. However, to our knowledge, there is little evidence to suggest that time of day effects on tissue properties were responsible for our results. On the other hand, there is substantial indirect evidence to suggest that endogenous modulation of upper airway neuromuscular function

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could have contributed to the effect of time of day on the critical closing pressure. Several studies have shown that skeletal muscle torque, strength and power are higher in the late afternoon compared with the morning (see Ref. 57 for review). These results have been obtained in a variety of muscles ranging from the adductor pollicis (24) to leg extensor muscles (47). Likewise, a decrease in the rate of tension development and one-half relaxation time has been reported in the morning compared with the evening and afternoon (24).

**Mechanisms responsible for the circadian modulation of upper airway collapsibility.** There are multiple inputs and stimuli (Fig. 7) presented on a nightly basis that could potentially be responsible for increases in the critical closing pressure from the evening to the morning, in individuals with sleep apnea. However, in the present study the elimination of sleep apnea with continuous positive airway pressure, combined with the employed experimental controls (i.e., maintenance of a supine position, similar sleep state), makes it unlikely that the time of day effect on the critical closing pressure was a consequence of 1) blunted feedback from upper airway receptors in response to inflammation, edema, and/or neural damage (4); or 2) enhanced chemoreceptor feedback in response to intermittent hypoxia (26, 28, 30, 51, 56). On the other hand, indirect evidence from animal studies indicate that endogenous modulation of upper airway motor neurons could be responsible for the increase in the critical closing pressure in the morning compared with the afternoon and evening. Serotonin, which is a neuromodulator of hypoglossal motor neuron activity, varies in a diurnal pattern (1, 31, 50). Likewise, Volgin et al. (52) showed that the endogenous excitatory drive to hypoglossal motor neurons may be altered through circadian mechanisms in part because of variations in the availability of 5-HT$_{2A}$ receptors, which was quantified at the mRNA and protein level.

The manner in which serotonin ultimately modulates hypoglossal or other upper airway motor neurons is unknown but one possibility could be linked to changes in metabolic rate, and more specifically to the modulation of carbon dioxide. Synaptic connections from serotonergic neurons in the raphe pallidus and obscurus project to hypoglossal motor neurons (23). These serotonergic neurons sense carbon dioxide levels (43), which increase genioglossus muscle activity in healthy humans during wakefulness (38, 39), and healthy humans (35, 44) or humans with obstructive sleep apnea (21) during non-rapid-eye-movement sleep. Thus, fluctuations in carbon dioxide via the modulation of raphe neurons could ultimately modulate upper airway muscle activity and collapsibility. Recently, we found that minute ventilation was reduced in the morning compared with the evening and afternoon coincident with the nadir in core body temperature and $P_{ETCO_2}$ (12). Coincident reductions in $P_{ETCO_2}$ and temperature were also noted in the morning in the present investigation. More importantly, decreases in the baseline partial pressures of carbon dioxide in the morning relative to the afternoon and evening were correlated to increases in the critical closing pressure, in support of the postulated role that carbon dioxide has in modulating upper airway collapsibility.

In addition to modulation of metabolic rate, circadian modulation of chemoreflex properties (Fig. 7) could also elicit fluctuations in carbon dioxide that promote increased upper airway collapsibility and ultimately alterations in breathing event frequency and/or duration. We recently discovered, using the experimental design employed in the present investigation, that chemoreflex sensitivity and the carbon dioxide reserve (i.e., the difference between the carbon dioxide that demarcates the point at which breathing is abolished and resting baseline levels of carbon dioxide) increased and decreased, respectively, during sleep in the morning compared with the evening and afternoon (12). In the presence of cyclic breathing events, increases in chemoreflex sensitivity and decreases in the carbon dioxide reserve could elicit profound hypocapnia and disfacilitation of upper airway motoneurons, increasing the propensity for upper airway collapse in the morning compared with the afternoon and evening. Likewise, given that the response to hypoxia is blunted or absent in the presence of hypocapnia (37, 40, 54, 55), it is possible that hypoxia does not significantly enhance receptor feedback before the abolition of hypocapnia, despite enhanced chemoreflex sensitivity. This outcome, coupled with the reduced metabolic rate in the morning, would delay the stimuli (i.e., hypoxia and hypercapnia) required to activate the effective recruitment threshold of upper airway motoneurons with the end result being an increase in breathing event duration.

**Physiological significance.** Increases in breathing event duration in the second half compared with the first half of the night in individuals with sleep apnea have been attributed to progressive diminution of feedback from upper airway sensory receptors in response to repeated trauma to pharyngeal tissues by upper airway vibration and closure (4). In addition, it has been proposed that progressive exposure to intermittent hypoxia and accompanying increases in chemoreflex sensitivity could contribute to increased breathing instability in the latter half compared with the initial half of the night. Thus it has largely been accepted that consequences of sleep apnea (i.e., intermittent hypoxia, inflammation) are responsible for altering
the complex of breathing events across the night (7, 22, 25). In addition to these possibilities, our published findings (12) and findings from the present investigation have shown that an endogenous rhythm may contribute to modification in breathing event characteristics. This effect may be mediated through a time of day effect on chemoreflex properties (i.e., chemoreflex sensitivity and carbon dioxide reserve) in addition to an inherent modulation of upper airway patency. Our findings lend credence to the possibility that consequences of sleep apnea are likely to interact with endogenous modulation of mechanisms that influence breathing instability. Our results may have important implications for the development of novel therapies to treat sleep apnea. More specifically, the effect of time of day on the administered dose of therapies targeted toward mitigating diminution of upper airway muscle function or increases in chemoreflex sensitivity and decreases in the carbon dioxide reserve in the morning in patients with sleep apnea must be considered.

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