Time of day affects chemoreflex sensitivity and the carbon dioxide reserve during NREM sleep in participants with sleep apnea

Mohamad El-Chami,1,2 David Shaheen,1,2 Blake Ivers,1,2 Ziauddin Syed,1,2 M. Safwan Badr,1,2,5 Ho-Sheng Lin,1,2,4 and Jason H. Mateika1,2,3

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 & 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 H, Mateika JH. Time of day affects chemoreflex sensitivity and the carbon dioxide reserve during NREM sleep in participants with sleep apnea. J Appl Physiol 117: 1149–1156, 2014. First published September 11, 2014; doi:10.1152/japplphysiol.00681.2014.—Our investigation was designed to determine whether the time of day affects the carbon dioxide reserve and chemoreflex sensitivity during non-rapid eye movement (NREM) sleep. Ten healthy men 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). Between sleep sessions, the participants were awake. During each sleep session, core body temperature, baseline levels of carbon dioxide (PETCO2) and minute ventilation, as well as the PETCO2 that demarcated the apneic threshold and hypocapnic ventilatory response, were measured. The nadir of core body temperature during sleep occurred in the morning and was accompanied by reductions in minute ventilation and PETCO2, compared with the evening and afternoon (carbon dioxide reserve: 21 ± 0.3 vs. 36 ± 0.5 vs. 3.5 ± 0.3 Torr, P < 0.002; hypocapnic ventilatory response: 2.3 ± 0.3 vs. 1.6 ± 0.2 vs. 1.8 ± 0.2 1·min−1·mmHg−1, P < 0.001). We conclude that time of day affects chemoreflex properties during sleep, which may contribute to increases in breathing instability in the morning compared with other periods throughout the day/night cycle in individuals with sleep apnea.

circadian rhythm; minute ventilation; intermittent hypoxia; inherent characteristics

CLINICAL STUDIES HAVE REPORTED that the number (8, 31) and duration of breathing events increase throughout the night (2, 4, 31), and that this increase is independent of sleep architecture and body posture. Similarly, a reduction in apneic events during daytime compared with nighttime sleep has been predicted by computer modeling simulations (36) and supported by experimental evidence, which showed that the apnea-hypopnea index was reduced during sleep in the day compared with the night in a small number of hypertensive men (30).

The potential mechanisms underlying the increase in apnea severity are likely multifactorial and phenotypically dependent (7, 25, 42). One possibility is that increases in chemoreflex sensitivity, coupled to a reduction in the carbon dioxide reserve (see METHODS, Data analysis, for definitions of chemoreflex sensitivity and carbon dioxide reserve), contributes to the progressive increase in breathing events across the night (17, 19). This observation is supported indirectly by Mahamed and colleagues (15), who reported that chemoreflex sensitivity to hypercapnia/hyperoxia increased during wakefulness in the morning, following 6 h of sleep, compared with the evening in sleep apnea participants. Sforza and colleagues (31) also reported that respiratory drive, measured as the rate of increase in esophageal pressure during apneic events, gradually increased throughout the night, even though the degree of oxygen desaturation and the rate of decrease during apneic events were constant. These findings, along with the established understanding that enhanced chemoreflex sensitivity promotes the occurrence of both central and obstructive breathing events (5–7, 17, 19, 41), provide support for the possibility that alterations in chemoreflex properties contribute to the promotion of breathing events at different points throughout the sleep period.

There are at least two possible mechanisms responsible for reported increases in chemoreflex sensitivity. The first is that exposure to intermittent hypoxia during sleep is responsible for the altered chemoreflex sensitivity during wakefulness that was reported to be increased in the morning compared with the evening in patients with sleep apnea (17, 19). This hypothesis is supported by our work and others, which have shown that exposure to intermittent hypoxia during wakefulness enhances chemoreflex sensitivity in healthy individuals (11, 16, 21, 39) and individuals with sleep apnea (10, 14). The second possibility is that alterations in chemoreflex properties reflect an endogenous oscillation that is linked to time of day. Indeed, Spengler et al. (33) and Stephenson et al. (37) have reported that chemoreflex sensitivity during wakefulness in healthy participants is modulated by a circadian rhythm. Despite these findings, no studies have examined if chemoreflex properties (i.e., apneic threshold, carbon dioxide reserve, and chemoreflex sensitivity) in sleep apnea participants are modulated during sleep according to the time of day and independent of intermittent hypoxia. The present investigation was designed to fill this void. Based on the published literature, we hypothesized that chemoreflex sensitivity would be greater and the carbon dioxide reserve reduced in the morning compared with measures obtained during sleep in the afternoon and evening.
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. Ten male participants with untreated pure or predominantly obstructive (i.e., a central component combined with an obstructive component) sleep apnea, but no other comorbidities (e.g., heart and lung disease, hypertension, and obesity), completed the protocol. Participants who completed the protocol visited the laboratory on six occasions. During the first visit to the laboratory, written informed consent was obtained, and thereafter, after a physical examination, health and lifestyle questionnaires, blood pressure and lung volume measures, along with a 12-lead ECG were completed. After ensuring that 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 into the protocol and given an actigraph watch (Actiwatch Spectrum, Philips Respironics, Murraysville, PA). The watch was used to monitor the sleep-wake schedule of the participants while they slept at home for 2 wk before obtaining physiological measurements on visits 4–6 (see subsequent paragraph for further details). We requested that the participants adhere to a regular sleep-wake schedule with a sleep onset time between 10–11 pm and a wake time of 7–8 am. We also requested that the participants avoid daytime napping. During the 2-wk period, the participants returned to the laboratory for a third visit. During this visit, continuous positive airway pressure was administered during sleep to determine the positive pressure required to maintain airway patency. In addition, a “practice trial” using the methodology and procedures required to determine the apneic threshold, chemoreflex sensitivity, and the carbon dioxide reserve was completed so that each participant experienced the required data collection methods and procedures before obtaining formal measurements.

After the 2-wk period, participants returned to the laboratory on three separate occasions (i.e., visits 4–6). Each of these visits was separated by a minimum of 7 days. We requested that the participants maintain a regular sleep-wake schedule during the 7-day interval that separated each visit. Details regarding two of the visits, along with the corresponding results, will be presented in separate publications. During one of these visits, core body temperature was measured over a 27-h period. One of the final three visits was designed to measure the corresponding results, will be presented in separate publications. During one of these visits, core body temperature was measured over a 27-h period. One of the final three visits was designed to measure the apneic threshold, chemoreflex sensitivity, and carbon dioxide reserve at three time points during the day/night cycle. Two days before these visits, participants were asked to abstain from alcohol and caffeinated beverages. On the day of the study, participants arrived at the laboratory at approximately 7:30 PM. On arrival, the participants ingested a radiotelemetry pellet (CorTemp Sensor, Palmetto, FL) to measure core body temperature every 10 s throughout the visit. This measure was used to establish the nadir of core body temperature and to confirm that this low point was similar to that measured during the study completed over 27 h. Following instrumentation, participants slept for 3 h during three separate sleep sessions (i.e., 10 PM–1 AM, 6–9 AM, and 2–5 PM). Subsequent to each sleep session, participants were placed in a semi-recumbent position during wakefulness. At the onset of each wake session (i.e., 1–6 AM and 9 AM–2 PM), 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 separated from sunlight and external cues including phones, clocks, radios, and television. The laboratory temperature was controlled at 22–23°C.

Interventions and procedures. During each sleep session, nasal noninvasive positive pressure mechanical ventilation was used to induce hyperventilation to determine the hypocapnic ventilatory response and the apneic threshold (Fig. 1A). Mechanical ventilation was applied for 3 min in a spontaneous-timed pressure support mode, as described in previous publications (18, 29). In this mode, a backup respiratory rate of 10 breaths/min was preset and timed breaths delivered if the participant’s respiratory rate fell below the set rate. During mechanical ventilation, the inspiratory positive airway pressure was increased gradually in 1- to 2-cmH2O increments at the beginning of each mechanical ventilation trial, while the expiratory positive airway pressure was fixed throughout mechanical ventilation at a pressure that eliminated apnea but maintained a reduction in airflow of 15–20% in each participant to prevent overdystension of the airway. Mechanical ventilation was terminated after 3 min, during expiration, by returning the inspiratory positive airway pressure to the baseline expiratory positive airway pressure. The ensuing hypocapnia resulted in either a hypopnea or central apnea (Fig. 1A). If an apnea was not induced, additional hyperventilation trials were completed until an apnea was evident, or arousal from sleep prevented the completion of additional trials. Central apnea was defined as an apneic event time ≥ 5 s. Each hyperventilation trial was separated by a 5-min period of baseline breathing.

Instrumentation. During sleep studies, the monitoring montage included an electroencephalogram (C3/A2, C4/A1, O1/A2, 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 RSS100-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, Ventura, CA) were obtained from air expired into sampling tubes attached to ports on the nasal mask. Nasal 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 digital 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, DaqTech Instruments, Akron, OH).

Data analysis. All polysomnography studies were analyzed for sleep stage, arousals, and respiratory-related events according to standard published criteria. The hyperventilation trials completed during the evening, morning, and afternoon sleep sessions were analyzed if associated with N2 or N3 of non-rapid eye movement sleep with an absence of arousal or ascent to N1. For each trial within a given session, the baseline period was represented by breaths recorded during the 2 min that immediately preceded the onset of mechanical ventilation. An average of the baseline periods measured during a given sleep session was calculated. The last five mechanically ventilated breaths before the ventilator was returned to a baseline expiratory positive airway pressure were averaged to represent the tidal volume achieved during the hyperventilation period. The change in the partial pressure of end-tidal carbon dioxide (PETCO2) was calculated as the difference between the PETCO2 recorded during the control period and the PETCO2 associated with the last five mechanically ventilated breaths. Minute ventilation was given a value of zero once a central apnea was induced.

The apneic threshold was defined by the PETCO2 associated with the occurrence of an apnea (Fig. 1B). The carbon dioxide reserve was
defined as the difference in PETCO2 measured during baseline breathing and the end-tidal partial pressure of carbon dioxide (PE TecO2) were measured, mechanical hyperventilation reduced carbon dioxide levels below baseline. Once mechanical ventilation ceased, an apnea was evident during recovery. B: a scatterplot obtained from one participant illustrating the minute ventilation response to stepwise reductions in PETCO2. In this example, reductions in PETCO2 occurred over 8 separate trials (C) until an apnea was achieved. The difference between baseline PETCO2 and the PETCO2 that demarcated the apneic threshold was considered to be the carbon dioxide reserve. The slope of the regression line fit to the data points was considered to be the chemoreflex sensitivity.

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 Student-Newman Keuls post hoc test was used to compare baseline levels of PETCO2 and minute ventilation, the PETCO2, that demarcated the apneic threshold, chemoreflex sensitivity, and the carbon dioxide reserve measured during the evening, morning, and afternoon sleep sessions. Data are presented as means, along with individual data points to signify variability. A P value ≤ 0.05 was considered statistically significant.

RESULTS

Table 1 shows the anthropometric variables obtained for the group. The results show that the participants were young and not obese, as indicated by the body mass index. The apnea/

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Values are means ± SE. AA, African-American.
The nadir of core body temperature was evident during the morning session (Fig. 2). Baseline measures of minute ventilation were lower in the morning compared with values in the evening ($P < 0.02$) and afternoon ($P < 0.02$) (Fig. 3A). A corresponding decrease in tidal volume (Fig. 3B), but not breathing frequency (Fig. 3C), was evident in the morning compared with the evening ($P < 0.04$) and afternoon ($P < 0.04$). Baseline $P_{ETCO_2}$ was reduced in the morning ($P < 0.002$) and afternoon ($P < 0.02$) compared with the evening (Fig. 4), while the $P_{ETCO_2}$ values that demarcated the apneic threshold were similar across the evening, morning, and afternoon sessions (Fig. 4). The carbon dioxide reserve was reduced in the morning ($2.1 \pm 0.25$ Torr) compared with the evening ($3.6 \pm 0.5$ Torr) ($P < 0.002$) and afternoon ($3.5 \pm 0.3$ Torr) ($P < 0.001$) (Fig. 4), while chemoreflex sensitivity was increased in the morning compared with the evening ($P < 0.001$) and afternoon ($P < 0.001$) (Fig. 3D).

**DISCUSSION**

We employed a constant routine protocol to measure chemoreflex properties during non-rapid eye movement sleep across the 24-h cycle in participants with sleep apnea. Our primary findings were that the carbon dioxide reserve was reduced and chemoreflex sensitivity was increased during sleep in the morning compared with the evening and afternoon. 

**Methodological considerations.** Our participants were relatively young and did not suffer from other comorbid conditions (i.e., diabetes, cardiovascular disease, and obesity). Thus the potential influence of these comorbidities on measures of chemoreflex sensitivity and the carbon dioxide reserve were controlled. The recruited participants were not typical of many...
patients seen in the sleep clinic, who are older, obese, and suffer from comorbid conditions. Consequently, the effect of time of day on chemoreflex properties may have a greater impact on breathing stability in a specific phenotype of sleep apnea patients whose disorder is less influenced by comorbidities and anatomical abnormalities. On the other hand, marked periodic breathing is evident following tracheostomy in sleep apnea patients with anatomically compromised airways (24). Thus our results may ultimately prove to be applicable to a number of phenotypes that comprise the sleep apnea population. Our study was also limited to investigating the effect of time of day on chemoreflex properties in men. Inclusion of only one sex was based on the availability of the participants to complete the protocol. Consequently, future studies are necessary to determine whether the effect of time of day on chemoreflex properties is sex dependent.

We obtained measures of the ventilatory response to hypocapnia; thus our findings do not necessarily reflect the effect that time of day has on chemoreflex sensitivity above resting carbon dioxide levels. Indeed, Katayama and colleagues (13) revealed in dogs that the ventilatory response to a reduction in carbon dioxide below resting carbon dioxide values was greater than the response to increases in carbon dioxide above resting values (see Figs. 4 and 5 in Ref. 13). Consequently, the role that time of day and chemoreflex properties has on respiratory drive during and immediately following an event may not be reflected in our results. Conversely, our findings directly reflect the ventilatory response to reductions in carbon dioxide that would typically occur in response to the induction of hypocapnia following hyperventilation that is principally responsible for perpetuating the cycle of breathing instability.

Baseline measures of ventilation, PETCO₂, temperature, and time of day. Previous studies have employed constant routine protocols to directly measure ventilation over a 24-h period of wakefulness in healthy humans breathing room air (1, 33, 38), or breathing air comprised of elevated levels of carbon dioxide (37) or reduced levels of oxygen (38). A circadian oscillation in minute ventilation (1, 33, 37) and its two components, tidal volume and breathing frequency (1), has been reported, although the circadian rhythm of minute ventilation approached but did not reach statistical significance in one investigation (33). The nadir of minute ventilation occurred in the early morning in the majority of studies (1, 37), but in one case was evident in the early evening (33). Our results are similar to published findings, since a significant reduction in minute ventilation and tidal volume was evident in the morning compared with the evening and afternoon.

Mortola suggested that circadian variations in minute ventilation in humans are mediated almost exclusively by changes in arousal state under the normal light-dark routine accompanied by fluctuations in sleep and wakefulness (22). However, our results, coupled with previous findings (1, 33, 37), indicate that the observed fluctuations in minute ventilation are in part influenced by the time of day, since fluctuations are evident under constant routine conditions when wakefulness and sleep are controlled. Although the fluctuation in minute ventilation is small, it is robust, since it remained evident, despite variability in the constant routine protocols employed in published studies (1, 33, 37) and the present investigation (e.g., 24 h of sleep deprivation vs. 3-h sleep sessions interspersed with wakefulness). Thus our results, coupled with previous findings, have established that minute ventilation varies according to the time of day during both non-rapid eye movement sleep and wakefulness in humans.

In addition to examining the effect of time of day on minute ventilation, simultaneous measures of core body temperature and PETCO₂ over a 24-h period of wakefulness, while breathing room air, has been measured in a few studies (33, 37, 38). In these studies, a well-established oscillation in core body temperature (12) was evident with the nadir occurring during the early morning hours (i.e., 6–8 AM) (33, 37, 38). However, the relationship between minute ventilation and core body temperature varied, with core body temperature in phase with minute ventilation in one investigation (37), but lagging 6–8 h in another (33). An obvious but small oscillation (≈1–2 Torr) in PETCO₂ has also been documented (20, 33, 38), and the relationship between minute ventilation and PETCO₂ was reportedly in phase (i.e., decreases in ventilation were accompanied by decreases in PETCO₂) with the nadir of the measures occurring between 6 PM and midnight (33). In agreement with previous findings, we showed in the present investigation that the nadir of core body temperature occurred in the early morning hours. Moreover, minute ventilation and PETCO₂ were significantly lower during sleep in the morning compared with the evening and afternoon. Given that we were not able to obtain continuous measures during sleep over the period of our investigation, we cannot state definitively that minute ventilation and PETCO₂ were in phase with the nadir of core body temperature. However, given our results, it is unlikely that the nadir of core body temperature coincides with the nadir of minute ventilation.

![Graph](image-url)  
**Fig. 4.** Combined histograms and scatterplots, which show the group mean and mean values for each participant, calculated from baseline measures of the PETCO₂ (group average indicated by top of the shaded rectangles, and individual data indicated by the solid circles), the PETCO₂ that demarcates the apneic threshold (group average indicated by bottom of the shaded rectangles, and individual data indicated by open circles), and the carbon dioxide reserve indicated by the vertical dashed line parallel to each histogram bar. Note that baseline PETCO₂ was reduced in the morning and afternoon compared with the evening (†), and that the carbon dioxide reserve was reduced in the morning compared with the evening and afternoon (††).
temperature lagged minute ventilation and $\text{PETCO}_2$ to the degree reported in a previous investigation (33).

It has long been established that $\text{PETCO}_2$ is influenced by arousal state, increasing during sleep compared with wakefulness (23, 28). However, our results, along with previous findings (20, 33, 38), confirm that, within a sleep or wake state, $\text{PETCO}_2$ is affected by the time of day. Moreover, our results reveal that a coincident reduction in minute ventilation and $\text{PETCO}_2$ occurred in conjunction with core body temperature. The mechanism(s) responsible for the coincident nadir in minute ventilation and $\text{PETCO}_2$ in the morning or the increase in minute ventilation and $\text{PETCO}_2$ in the other sessions is unknown. Given the increase in chemoreflex sensitivity (see Chemoreflex properties and time of day for further discussion) that we measured in the morning, compared with the afternoon and evening, it seems unlikely that chemoreflex inputs were principally responsible for the coincident decrease in ventilation and $\text{PETCO}_2$ observed in our investigation. This finding is intriguing, since it is generally accepted that minute ventilation during non-rapid eye movement sleep is controlled solely by input from the chemoreflexes in healthy humans (26). Moreover, given the coincident variations in core body temperature, minute ventilation, and $\text{PETCO}_2$ in the present investigation, coupled with similar published findings that included measures of metabolic rate during wakefulness (33), a robust link between minute ventilation and metabolism is apparent. Despite this result, the mechanism responsible for the link between metabolism and minute ventilation remains unknown. Perhaps parallel inputs from the hypothalamus and the preoptic area, which receive projections from the suprachiasmatic nucleus to the respiratory controller and temperature regulation network, are responsible for the coincident reduction in minute ventilation, $\text{PETCO}_2$, and temperature that was observed. Indeed, activation of the nucleus tractus solitarius and ventrolateral medulla in rats induces a coincident decrease in temperature and $\text{PETCO}_2$ (3). Similarly, a variety of endocrine mechanisms (e.g., cortisol, melatonin) might also contribute to coincident variations in minute ventilation, $\text{PETCO}_2$, and temperature (33, 35).

Chemoreflex properties and time of day. A number of studies have examined the effect of time of day on chemoreflex properties in healthy humans during wakefulness (9, 15, 27, 32, 33, 37). Some studies were designed to measure the threshold and/or chemoreflex sensitivity in the evening, before sleep, and in the morning, immediately after sleep (9, 15). The results were variable in that the ventilatory response to increases in carbon dioxide during wakefulness was reported to be unchanged (9) or altered as a consequence of a decrease in the chemoreflex threshold (15) in the morning compared with the evening. Differences in the methodology used to measure chemoreflex properties and the composition of populations recruited for each study could account for some of the variability (9, 15). Nonetheless, despite this variability, the reported change (15) or lack thereof (9) would tend to promote the maintenance of breathing stability across the evening to morning transition. Other studies have measured chemoreflex properties in healthy humans over a 24-h period (27, 32, 33, 37). In two studies, the participants were awake and inactive throughout the constant routine protocol (33, 37). In the remaining studies, measurements were also made during wakefulness (27, 32); however, participants were allowed to either sleep at will (27) or sleep for 2 h from 4–6 AM in addition to engaging in moderate physical activity (32). Results from these studies revealed that a circadian oscillation in the ventilatory response to isocapnic hypoxia (27, 32) or the ventilatory response to carbon dioxide in the presence of normoxia (27, 33) or hypoxia (37) was evident. The acrophase of the ventilatory response to isocapnic hypoxia reportedly occurred at noon and remained relatively constant at other times throughout the 24-h cycle (32). Inclusion of exercise and one period of sleep in the constant routine protocol may have influenced the results (32). Spengler and Shea (33) reported that the acrophase of the ventilatory response to carbon dioxide (i.e., as a result of an increase in chemoreflex sensitivity) was evident between the hours of 10 AM and 2 PM, while the bathyphase, which was evident starting at 6 PM remained stable for 12–14 h. In other studies, the acrophase of the ventilatory response to carbon dioxide, either as a consequence of an increase in the chemoreflex threshold (37) or a decrease in chemoreflex sensitivity (27), was evident later in the evening (6 PM), while the onset of the bathyphase was manifested in the morning (27, 37). Although some discrepancies exist between studies, collectively the results suggest that the ventilatory response to hypoxia or carbon dioxide may remain relatively stable or decrease across the hours that are normally associated with sleep in healthy humans.

To our knowledge, only two studies have explored the effect of time of day on chemoreflex properties in participants with sleep apnea (9, 15). In both studies, measures of the chemoreflex threshold (15) and/or chemoreflex sensitivity (9, 15) were obtained during wakefulness in the evening, before sleep, and in the morning, immediately after sleep (9, 15). Mahamed and colleagues (15) reported that the chemoreflex sensitivity to hyperoxia/hypercapnia was increased in the morning compared with the evening, while Fuse et al. (9) reported that no changes in chemoreflex sensitivity were evident. Mahamed and colleagues (15) suggested that the reasons for the discrepant findings was that Fuse et al. (9) used a standard hyperoxic rebreathing technique that measures ventilatory responses in the hypercapnic range of 50–80 mmHg, whereas Mahamed and al. (15) used a modified rebreathing technique that measures ventilation in the normocapnic range of 35–60 mmHg. Independent of this difference, the design of both studies eliminated the possibility of examining the effect of time of day on chemoreflex properties in humans with obstructive sleep apnea during sleep. More specifically, measures were not obtained during sleep; as such, the measures of chemoreflex properties during wakefulness in the morning were likely impacted by stimuli linked to breathing events (e.g., arousal, intermittent hypoxia) that occurred during the preceding sleep period (9, 15). Our primary findings filled this void and revealed that the ventilatory response to hypocapnia was significantly enhanced, while the carbon dioxide reserve was reduced, in the morning compared with the evening and afternoon. Conversely, the $\text{PETCO}_2$ that demarcated the apneic threshold remained stable across the three sleep sessions. Given this stability, the reduction in the carbon dioxide reserve in the morning was due principally to a reduction in baseline carbon dioxide. Thus a fundamental alteration in chemoreflex properties linked solely to the time of day might exist in sleep apnea participants compared with healthy humans, if our analysis of the published results from healthy humans is correct (see preceding paragraph for further discussion).
Physiological significance. A few clinical studies have reported that the number (8, 31) and duration (2, 4, 31) of breathing events increase throughout the night, independent of sleep architecture and body posture. Similarly, a reduction in apneic events during daytime compared with nighttime sleep has been predicted by computer modeling simulations (36) and supported by experimental evidence, which showed that the apnea-hypopnea index was reduced during sleep in the day compared with the night in a small number of hypertensive men (30).

The duration of breathing events might be increased as a consequence of diminished feedback from upper airway sensory receptors which Cala and colleagues (2) have suggested is due to repeated trauma to pharyngeal tissues as a consequence of upper airway vibration and closure. In parallel, an increase in breathing instability and the number of events from the beginning to the end of the night may be driven by an increase in chemoreflex sensitivity and a reduction in the carbon dioxide reserve, respectively (17, 19). Indeed, our findings support this suggestion, since chemoreflex sensitivity was increased and the carbon dioxide reserve decreased in the morning compared with the afternoon and evening.

One of the stimuli that could initiate increases in chemoreflex sensitivity is intermittent hypoxia, a hallmark of sleep apnea (17, 19). Indeed, numerous studies reported that exposure to intermittent hypoxia during wakefulness increases the ventilatory response to carbon dioxide and sustained hypoxia (10, 14, 16, 21, 39). However, questions remained regarding whether or not chemoreflex properties were altered over time during sleep in participants with sleep apnea. Our results have partially filled this void, since we established that chemoreflex sensitivity is increased and the carbon dioxide reserve was decreased in the morning compared with the afternoon and evening. We also ascertained that these alterations were linked to the time of day, independent of exposure to intermittent hypoxia. Thus progressive increases in chemoreflex sensitivity during sleep in participants with sleep apnea could, in part, be the cause and not the consequence of breathing events. Our findings may have important implications for the development of novel therapies to treat sleep apnea. More specifically, the findings may have important implications for the development of novel therapies to treat sleep apnea. More specifically, the findings may have important implications for the development of novel therapies to treat sleep apnea. More specifically, the findings may have important implications for the development of novel therapies to treat sleep apnea. More specifically, the findings may have important implications for the development of novel therapies to treat sleep apnea. More specifically, the findings may have important implications for the development of novel therapies to treat sleep apnea.

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