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Circadian Modulation Of Breathing Stability And Respiratory Plasticity

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CIRCADIAN MODULATION OF BREATHING STABILITY AND RESPIRATORY PLASTICITY

by

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DISSERTATION

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Advisor Date

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Co-Advisor

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DEDICATION

This dissertation is dedicated to my Family.

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I would like to express my deepest gratitude to my advisor Dr. Jason Mateika, who has supported me throughout this journey. I cannot thank him enough for his patience, guidance, and immense knowledge. Because of you I leave this lab a better scientist, and a better man.

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PREFACE - SPECIFIC AIMS

Sleep apnea is characterized by a decline in breathing stability, leading to a decrease or complete cessation of airflow during sleep. The occurrence of adverse breathing events tends to be cyclical, the severity of which is affected by alterations in chemoreflex properties and upper airway collapsibility. The accompanying intermittent hypoxia and sleep fragmentation associated with breathing events has been linked to many cardiovascular, cognitive and metabolic disorders. Published studies indicate that the severity of breathing events is modulated by an endogenous circadian rhythm; since the duration and number has been reported to increase from the evening to the morning, while computer simulations predicted a decrease in such events during daytime compared to nighttime sleep.

Aim 1 *is designed to confirm that the severity of breathing events during sleep is affected by the time of day. Aim 1 will also investigate if alterations in chemoreflex properties and upper airway collapsibility are responsible for the alterations in breathing events across the 24 hour cycle. We hypothesize that the duration and number of breathing events will be greater during sleep in the morning compared to the afternoon and evening. We also postulate that the critical closing pressure (i.e. a measure of upper airway collapsibility) and chemoreflex sensitivity will increase, while the carbon dioxide reserve will decrease in the morning compared to the afternoon and evening.*

If breathing stability and the mechanisms that affect this stability are altered by the time of day this could impact on therapeutic treatments for obstructive sleep apnea. For example, continuous positive airway pressure, which is used to treat sleep apnea, could be reduced at certain times of the day/night if those mechanisms that influence upper airway collapsibility are affected. Likewise, if other concurrent mechanisms that promote breathing stability are affected by the time of day this could lead to the development of adjunct therapies that could be used

independently or alongside continuous positive airway pressure to more effectively eliminate breathing events. We, and other investigators, have shown that an increase in upper airway muscle activity is initiated in response to intermittent hypoxia. Increases in upper airway muscle activity would likely promote stability and contribute to decreasing the severity of apneic events.

Aim 2 *will investigate whether the therapeutic continuous positive airway pressure required to eliminate breathing events varies across the 24 hour cycle. We hypothesize that this pressure will be highest in the morning as a result of the heightened breathing instability and lowest during sleep in the afternoon and early evening where the mechanisms promoting instability are mitigated. In addition, Aim 2 will examine if initiation of respiratory plasticity, namely long-term facilitation of upper airway muscle activity, following exposure to intermittent hypoxia impacts the therapeutic pressure and is affected by the time of day. We hypothesize that long-term facilitation of upper-airway muscle activity will be promoted in the evening and afternoon in line with the alterations in the aforementioned mechanisms that enhance breathing stability, and mitigated in the morning where breathing is less stable.*

Our findings could provide the rationale for careful determination of therapeutic pressure during sleep over the day/night cycle. Our results will also establish if other potential novel adjunct therapies (i.e. intermittent hypoxia) might serve to improve the treatment of sleep apnea. Lastly, our findings will provide the rationale for altering the administered dose of treatments at that have recently been proposed to treat sleep apnea at specific time points.

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CHAPTER 1 - INTRODUCTION

Sleep disordered breathing

Sleep serves an important function in many species, including humans; from physiological maintenance of cells, to memory consolidation and other cognitive functions. However sleep poses a challenge for the respiratory system. During wakefulness the level of carbon dioxide and the consequent intracellular pH are the principal stimuli for ventilation (Fink, 1961); however other neural mechanisms also contribute to the maintenance of ventilation (Longobardo *et al.*, 2002). Environmental factors, including audio and visual stimuli, as well as mental activity, modulate breathing independent of changes in blood gas levels and chemoreceptor feedback (Fink, 1961; Shea *et al.,* 1987; Nielsen and Smith, 1952; Mohan and Duffin, 1997). In contrast, during sleep, breathing is controlled primarily by the carotid body and central chemoreceptors located in the ventral region of the brainstem. Changes in blood gases levels detected by these chemoreceptors induce appropriate changes in ventilation to preserve normal levels (Mateika and Syed, 2013). In addition to the absence of the wakefulness drive, the activity of upper airway dilator muscles decreases at sleep onset, rendering a narrower airway with a greater tendency to collapse and create airflow obstruction.

Apnea during sleep is defined by the complete cessation of breathing for a period of at least 10 seconds. Both apneas and hypopneas (i.e. partial reductions in ventilation) lead to a decrease in oxygen and an increase in carbon dioxide levels in the blood. The cessation of airflow can be due to collapse of the upper airway, which is defined as an obstructive apnea, or the cessation of respiratory motor output from the brainstem, which is characteristic of a central apnea. An apnea can also be of mixed nature, which includes a central and obstructive component (Dempsey *et al*., 2010). Risk factors for sleep disordered breathing include body weight, gender, age, and craniofacial structure. Untreated sleep apnea/hypopnea increases the

risk of fatal and non-fatal cardiovascular events (Marin *et al.*, 2005, Dempsey *et al,* 2010). Moreover, disorders linked to insulin resistance and altered neurobehavioral function could be directly or indirectly linked to sleep apnea; since recurrent exposure to hypoxia, hypercapnia and sleep disruptions affects many systems and cellular processes (Dempsey *et al.*, 2010). These processes include activation of pro-inflammatory pathways, increases in reactive oxygen species, and disruptions in hormonal levels (e.g. leptin and ghrelin). Consequently, obstructive sleep apnea likely plays a role in the initiation and perpetuation of many disorders.

Mechanisms affecting the severity of sleep disordered breathing

Apneic events tend to occur in a cyclical fashion (Figure 1). At the onset of sleep the upper airway collapses, leading to an apneic event and subsequent changes in blood gas levels. The origins of upper airway collapse are two-fold. Isono *et al.* (1997) observed that the upper

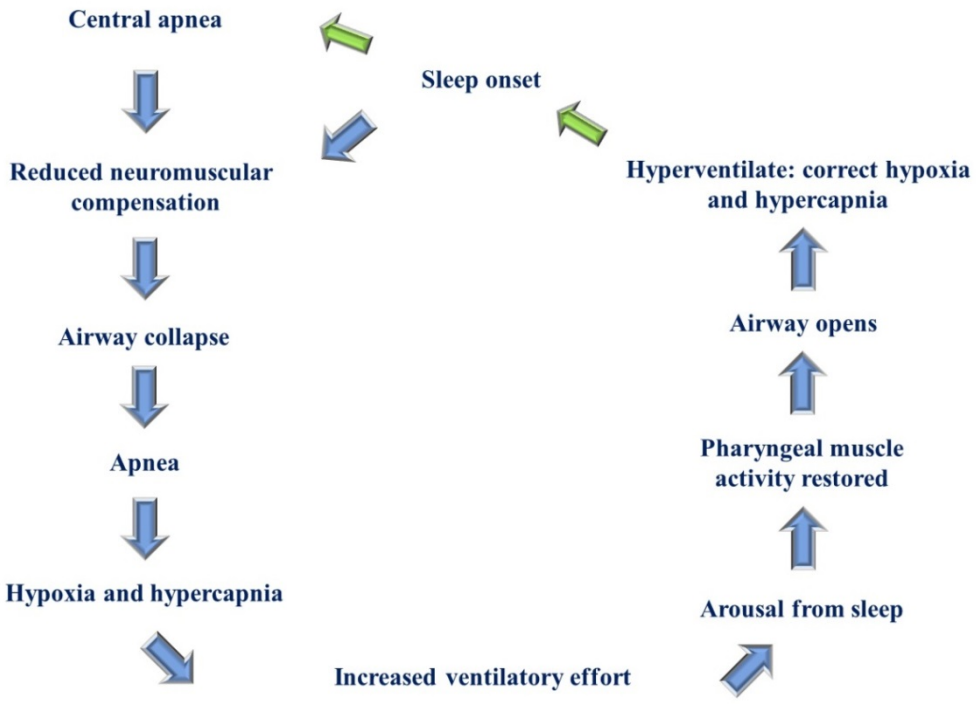


Figure 1. Schematic diagram showing the sequence of events leading to the development of a central and/or obstructive apnea, and subsequent events that ultimately results in re-establishing patency of the upper airway. (Mateika and Syed, 2013)

airway was more collapsible in sleep apnea patients compared to controls in the presence of complete neuromuscular blockade, which suggests that tissue properties have a role in upper airway collapsibility. In addition, it was observed in both animal and human models that upper airway collapsibility is increased during anesthesia or sleep in response to reductions in neuromuscular activity (Brouillette *et al.*1979; Seelagy *et al.* 1994; Patil *et al.* 2007 and McGinley *et al.* 2008). Younes *et al.* (2003) suggested that one third of apneic events can be attributed to structural alterations and defects, while the remainder is attributed to neuromuscular deficits.

Independent of the mechanism, apneic events are usually accompanied by arousal from sleep which restores airway patency. However, arousal is often associated with increases in ventilation, which are meant to restore normal blood gases levels, but often exceed metabolic rate leading to hypocapnia. While such a decrease is not necessarily problematic in the wake state, it is during sleep. If carbon dioxide decreases below a set point deemed "the apneic threshold", a central apnea will occur once sleep is re-established. In addition, the decrease in carbon dioxide is accompanied by a reduction in upper airway neuromuscular compensation, since carbon dioxide is an essential stimulus in the modulation of upper airway muscle activity via the hypoglossal nerve. The cycle then repeats itself multiple times throughout the night (Mateika and Syed, 2013).

The severity of this cyclical pattern depends upon multiple mechanisms, including, but not exclusive to, upper airway collapsibility and properties of the chemoreflex response to changes in blood gases. As seen in Figure 1, reduced neuromuscular compensation will decrease upper airway patency and promote the occurrence of an apnea. In addition, heightened chemoreceptor sensitivity, to decreases in oxygen and increases in carbon dioxide levels that occur during breathing events, promotes exaggerated levels of ventilation upon arousal which

increases apneic severity by inducing hypocapnia. Reductions in the carbon dioxide reserve (i.e. the difference between the carbon dioxide baseline level and the apneic threshold), whether a consequence of changes in chemoreflex sensitivity, the apneic threshold or baseline carbon dioxide, promote hypocapnia.

Time of day affects breathing mechanisms

Circadian effect on breathing instability. In addition to apneic events occurring in a cyclical fashion, the severity of these events, characterized by an increase in number and duration of events, appears to be affected by the time of day. A number of studies have shown that breathing events increase in number (Fanfulla *et al.* 1997) and duration (Cala *et al,* .1996; Sforza *et al.,* 1998) during sleep from the beginning to the end of the night independent of sleep stage. In addition, computer modeling simulations have predicted a reduction in the number of breathing events during daytime compared to nighttime sleep (Stephenson *et al.*, 2004). If the results of these small clinical studies are correct then fluctuations in chemoreflex properties (i.e. chemoreflex sensitivity and a reduction in the carbon dioxide reserve) and mechanisms that influence upper airway collapsibility across the 24 hour cycle could be responsible for the reported increases in breathing instability.

This suggestion is supported by the results obtained by Sforza *et al.* (1998) who showed that an increase in the duration of breathing events during the night was accompanied by an increase in respiratory drive, which could be a consequence of enhanced chemoreflex sensitivity. In addition to these findings, Mahamed *et al*. (2005) measured chemoreflex sensitivity during wakefulness before and after 6 hours of sleep in individuals with sleep apnea and reported an increase in the morning compared to the evening. Therefore, increases in chemoreflex sensitivity are evident from the evening to the morning in individuals with sleep apnea. However, these increases may not be a consequence of time of day *per se* but rather a

consequence of confounding factors that are linked to sleep apnea, including intermittent hypoxia. Indeed, numerous studies have shown that chemoreflex sensitivity increases following exposure to intermittent hypoxia in both healthy individuals and individuals with sleep apnea. However, to our knowledge no studies have been designed to determine if alterations in chemoreflex properties are affected by time of day independent of other confounding factors associated with sleep apnea. Our preliminary results (see Figure 4 in Chapter 2) suggest that this is the case, since the carbon dioxide reserve was reduced and chemoreflex sensitivity was increased in the morning compared to the evening and afternoon, independent of other confounding factors, in a group of sleep apnea participants that completed a constant routine protocol. *Therefore, Aim 1 of my prospectus is designed in part to confirm that the severity of sleep apnea is affected by the time of day. Likewise, aim 1 is designed to determine if chemoreflex properties are affected by the time of day independent of confounding factors that normally are linked to breathing instability during sleep.*

Moreover, time of day might also affect upper airway muscle function. This suggestion is supported indirectly from studies (Martin *et al.* 1999 and Zhang *et al.* 2009) which reported that the force and strength of limb muscles are greater in the late afternoon compared to the early morning, which could be indicative of a circadian rhythm to the control of muscle activity. This possibility is supported by studies which have shown that serotonin (Agren et al., 1986, Mateos et al., 2009 and Sun et al., 2002), brain derived neurotrophic factor (BDNF) and phosphorylated extracellular regulated kinases (Serchov and Heumann, 2006), which all have a role in modulating hypoglossal motor neuron activity, are regulated by a circadian rhythm. In addition, 5-HT_{2A} receptor mRNA levels in rats differ between two time points, being higher at the onset of the active phase (6–7 pm) compared to the onset of the rest period (8–9 am) (Volgin et al. 2013). To my knowledge, no studies have examined the variation in upper airway collapsibility across

the 24 cycle. However, our preliminary data (see Figure 6 in Chapter 2) indicates that the active critical closing pressure of the upper airway, which is an indicator of the neuromuscular contribution to maintaining airway patency, is more positive (i.e. more collapsible) in the morning compared to the evening and afternoon.

Therefore, Aim 1 will also examine if passive tissue and neuromuscular properties that influence airway patency is affected by the time of day in individuals with sleep apnea. Collectively, we hypothesize that the severity of apneic events will be greater in the morning compared to the afternoon and evening. Moreover, we hypothesize that chemoreflex sensitivity will be greater, the carbon dioxide reserve will be reduced and the airway will be more collapsible in the morning compared to the afternoon or evening sleep.

Respiratory plasticity

Increases or decreases in neuronal activity can strengthen or weaken connections as well as activating *de novo* and/or pre-existing neuronal pathways that have been quiescent. This phenomenon is often referred to as synaptic plasticity. Forms of synaptic plasticity include respiratory long-term facilitation which is characterized by a progressive increase in respiratory motor output during normoxic periods after exposure to intermittent hypoxia, and progressive augmentation which is characterized by enhancement of the hypoxic ventilatory response (Mateika and Syed, 2013).

Long-term facilitation is initiated by intermittent but not sustained hypoxia (Figure 2). Single hypoxic episodes that are brief can lead to acute increases in respiratory motor output that return to baseline levels within minutes following the hypoxic episode. However, successive episodes (i.e. intermittent hypoxia) lead to sustained respiratory motor output for up to 90 minutes following the final hypoxic episode (Figure 2) (Baker and Mitchell, 2000; Mitchell et al., 2001).

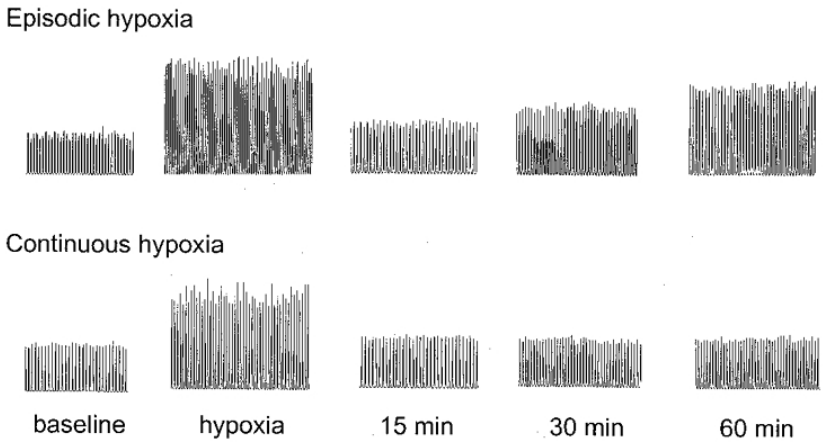


Figure 2. Integrated phrenic discharge in one rat exposed to episodic hypoxia and one rat exposed to 20 min continuous hypoxia. Integrated phrenic discharge during baseline, hypoxia, and 15, 30 and 60 min post-hypoxia. Note that only intermittent hypoxia elicited LTF. (Baker and Mitchell, 2000)

This phenomenon is governed by cellular mechanisms that respond differently to changes in the intensity of administered hypoxia. Exposure to moderate acute intermittent hypoxia leads to the release of serotonin at the level of spinal respiratory motoneurons. The binding of serotonin to 5-HT_2 receptors leads to production of brain derived neurotrophic factor, activation of TrkB receptor and ERK-MAPK signaling that results in facilitation of the input to the respiratory motor neuron (Figure 3a). Also contributing to this pathway is the production of reactive oxygen species by NADPH oxidase which inhibits protein phosphatases and leads to activation of PKC and production of BDNF. This pathway is referred to as the Q pathway since it involves activation of the G_q protein coupled to the 5-HT_2 receptor. In response to exposure to severe intermittent hypoxia (Figure 3a), there is accumulation of extracellular adenosine which activates adenosine 2A receptors and consequently TrkB and Akt, with eventual facilitation of the motor input. This pathway is deemed the S pathway since it involves activation of the the G_s protein coupled to the adenosine 2A receptor. Under conditions of severe hypoxia the S pathway may exert an inhibitory effect on the Q pathway thereby dominating the control of the response. This cross talk between the 2 pathways allows for great flexibility in the system and the ability to express different forms of plasticity in response to different conditions.

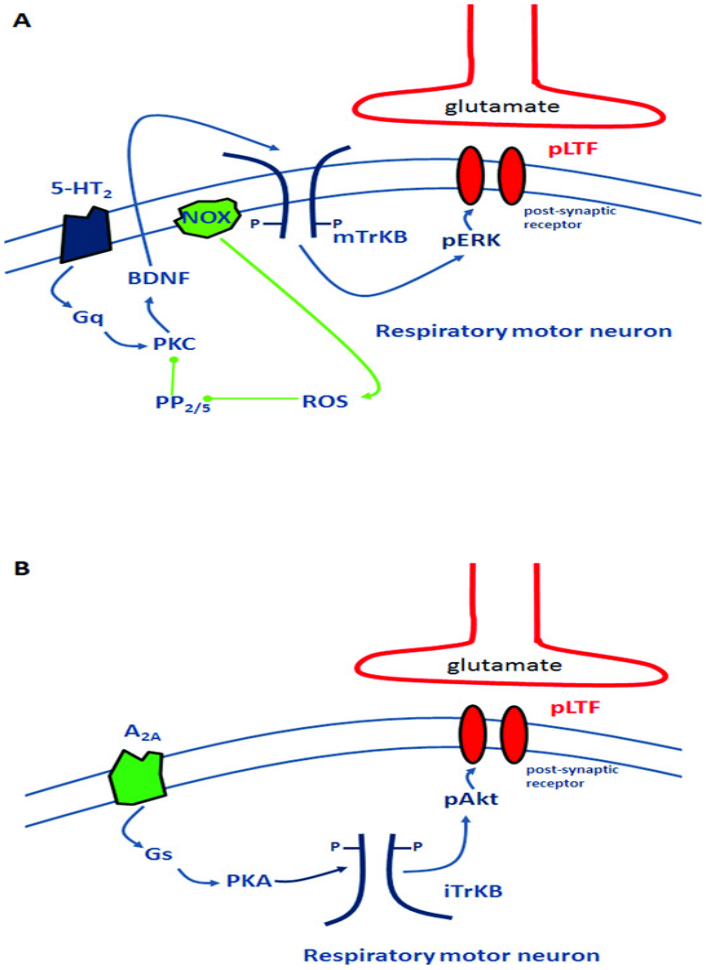


Figure 3a. Cellular pathways involved in phrenic motor long term facilitation following acute intermittent hypoxia. (A) The Gq protein–coupled 5-HT2 receptor is activated leading to PKC activation and synthesis of brain derived neurotrophic factor. The activation of TrkB by BDNF phosphorylates ERK, thereby facilitating input to the motor neuron. (B) The Gs protein–coupled adenosine 2A (A2A) receptor is activated, leading to a cascade of PKA, TrkB and Akt activation. The result is the facilitation of respiratory motoneuron input. (DeVinney et al., 2013)

Long-term facilitation of ventilation and genioglossus muscle activity have been documented in healthy humans and humans with obstructive sleep apnea during both wakefulness and sleep. We have shown in our laboratory that this phenomenon is exquisitely dependent on carbon dioxide levels. In other words, long-term facilitation manifests itself when carbon dioxide is sustained slightly above baseline levels but is not evident when carbon dioxide levels are not controlled and hypocapnia ensues. The presence of this phenomenon in humans is exciting because it may ultimately lead to the development of adjunctive therapies to treat obstructive sleep apnea.

Presently, the most common treatment for sleep apnea is continuous positive airway pressure. The positive pressure serves as a splint to maintain airway patency, thereby relieving

airflow obstruction. However, there are multiple side effects associated with this treatment modality that has resulted in limited compliance. The lack of compliance in some cases may be a consequence of elevated levels of positive pressure that are required to maintain airway patency. Thus, adjunctive therapies that contribute to reducing the effective positive airway pressure may ultimately lead to improved compliance. Recognition that stability of the upper airway is influenced by the time of day (see above Specific Aim 1) may lead to careful determination of therapeutic positive pressure values during sleep at different times across the 24 hour cycle. Moreover, if long-term facilitation of genioglossus muscle activity can be initiated in individuals with sleep apnea this phenomenon may serve to reduce the effective therapeutic pressure to treat sleep apnea at a given point in the 24 hour cycle. More interestingly, if this form of respiratory plasticity is affected by the time of day, its influence may be more effective at certain points throughout the day/night cycle.

As a result, *Aim 2 is designed to determine if the continuous positive airway pressure required to eliminate breathing events varies across the 24 hour cycle, hence significantly impacting the therapeutic approach to the treatment of sleep apnea. Aim 2 will also determine if long-term facilitation of upper airway muscle activity influence levels of therapeutic positive airway pressure and whether this influence is variable and dependent on the time of day.*

CHAPTER 2 - TIME OF DAY AFFECTS THE FREQUENCY AND DURATION OF BREATHING EVENTS AND THE CRITICAL CLOSING PRESSURE DURING NREM SLEEP IN PARTICIPANTS WITH SLEEP APNEA

(This chapter contains previously published material. See Appendix B)

Introduction

Clinical studies have reported that independent of sleep stage and body position, the number (Fanfulla *et al.*, 1997;Sforza *et al.*, 1998) and duration (Cala *et al.*, 1996;Charbonneau *et al.*, 1994;Lavie *et al.*, 1981;Montserrat *et al.*, 1996a;Sforza *et al.*, 1998) 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 to nighttime sleep may occur (Stephenson, 2004). This latter postulation has been reported in a small number of hypertensive men, although other confounding factors may have influenced the findings (Scharf *et al.*, 1990).

The potential mechanisms underlying the increase in the number and duration of breathing events across a 24-hr period are likely multifactorial and phenotypically dependent. One possibility is that increases in chemoreflex sensitivity, coupled to a reduction in the carbon dioxide reserve contribute to the progressive increase in breathing events across the night (*see Chapter 3 for the detailed findings*) (El-Chami *et al.*, 2014a;Mateika & Narwani, 2009;Mateika & Sandhu, 2011;Mateika & Syed, 2013a;Mateika, 2015;Mateika *et al.*, 2015). This possibility is well founded in that enhanced chemoreflex sensitivity promotes the occurrence of both central and obstructive breathing events (Dempsey *et al.*, 2004;Dempsey, 2005;Dempsey *et al.*, 2010;Yokhana *et al.*, 2012). 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 (Chowdhuri *et al.*, 2010;Gerst, III *et al.*, 2011;Griffin *et al.*,

2012;Khodadadeh *et al.*, 2006;Mateika *et al.*, 2004a;Wadhwa *et al.*, 2008). However, we showed that increases in chemoreflex sensitivity and decreases in the carbon dioxide reserve were evident during sleep in the morning compared to the evening and afternoon; independent of physiological hallmarks typically associated with sleep apnea (i.e. intermittent hypoxia and sleep fragmentation) (El-Chami *et al.*, 2014a).

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 (Berry *et al.*, 1995;Cala *et al.*, 1996;Montserrat *et al.*, 1996a) 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 frequency of apnea events since collapsibility of the upper airway could be impacted by upper airway muscle fatigue associated with exposure to intermittent hypoxia (Bradford *et al.*, 2005;McGuire *et al.*, 2002;Ray *et al.*, 2007) and dampening of excitatory sensory inputs from the upper airway receptors (Cala *et al.*, 1996).

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 [see (Zhang *et al.*, 2009) for review] have shown that force, along with other measures of muscle function [i.e. rate of tension development and one-half relaxation time (Martin *et al.*, 1999)], in skeletal muscle ranging from the abductor pollicus (Martin *et al.*, 1999) to leg extensor muscles (Sedliak *et al.*, 2008), are enhanced in the late afternoon and evening compared to 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 sub-types (i.e. $5HT_{2A}$) are modulated by the time of day (Agren *et al.*, 1986;Mateos *et al.*, 2009;Sun *et al.*, 2002;Volgin *et al.*, 2013).

Therefore, the primary aims of the present investigation were two-fold. 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 hour cycle. We hypothesized that the duration and frequency of breathing events would be greater during sleep in the morning compared to 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 to 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 (Figure 3). During the first visit to the laboratory, written informed consent was obtained and thereafter a physical examination, health and lifestyle questionnaires, blood pressure, lung

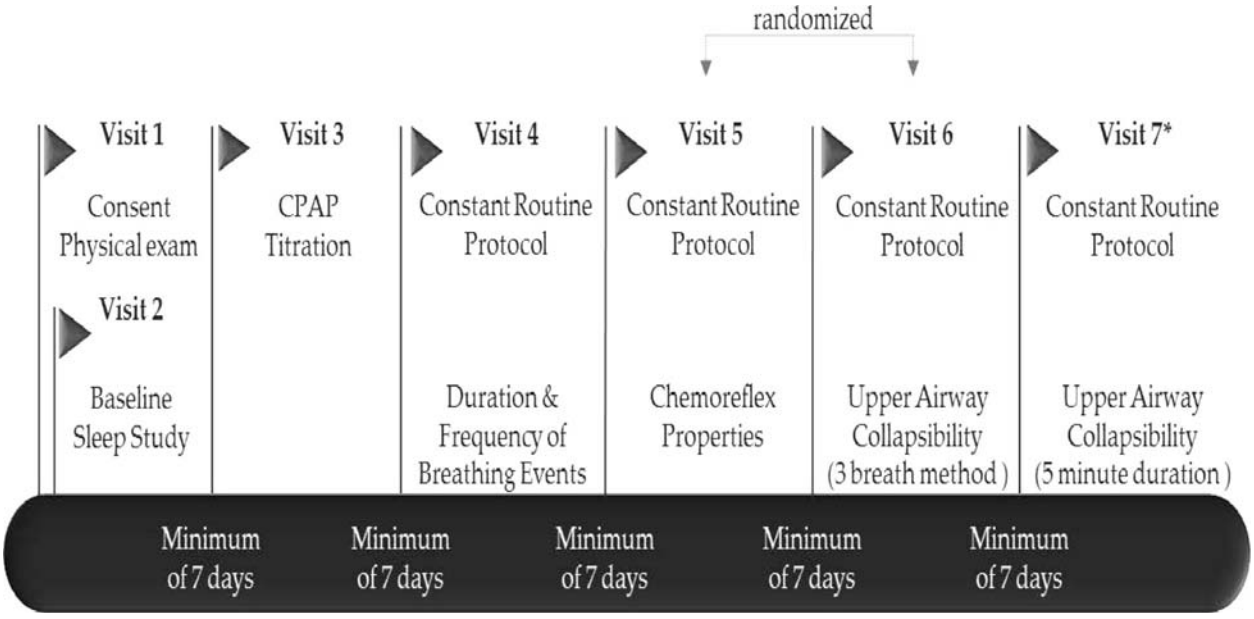


Figure 3. A schematic diagram showing the protocol completed by the participants. * - 8 of 13 participants completed visit 7.

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 weeks, using an actigraph watch (Actiwatch Spectrum, Philips Respironics, Murraysville, PA), before obtaining the planned physiological

measurements on visits 4–6 (*see* subsequent paragraph for further details). During the two week time period 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 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 week time period, participants returned to the laboratory on three separate occasions (i.e., *visits 4 –6*). Each visit was separated by a minimum of seven 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 seven day interval that separated each visit. Participants were asked to abstain from alcohol and caffeinated beverages at least one day prior to the visit. On the day of each study, participants arrived at the laboratory at approximately 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 hours 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 semi-recumbent 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 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 the 4th visit, breathing events (i.e. apneas and hypopneas) were measured during each sleep session. Measures of breathing events were obtained from 12 of 13 participants. One participant expressed a desire to withdraw from the study because of time constraints. Consequently, we chose to obtain measures of the critical closing pressure (Figure 4) rather than measures of breathing events, prior to withdrawal. On the 5th and 6th visits, which were randomized, chemoreflex properties (i.e., chemoreflex threshold, sensitivity and the carbon dioxide reserve) or the upper airway critical closing pressure was measured during each sleep session in 10 participants. A description of the methods used to measure the chemoreflex properties, and the results which demonstrated the effect of time of day on these properties can be found in Chapter 3, in addition to being published previously (El-Chami *et al.*, 2014a). Subsequent to this publication, 3 additional participants were enrolled, so that measures of the critical closing pressure were obtained from thirteen participants.

In order 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 (Figure 4). During each session, airway patency was initially maintained using a holding pressure that was 1.7 ± 0.5 cmH_2O less than the therapeutic pressure determined during visit 3. The holding pressure generated a reduction in airflow of 10–15% and was employed to prevent over-distension 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 cmH_2O (Pcrit 3000, version 1.0, Philips Respironics, Murrysville, PA) for a duration of 3 to 5 breaths. Each step down in pressure was separated by a 1 minute recovery period at the holding pressure. Stepwise reductions in pressure continued until the airway was fully collapsed, which was defined by measures of airflow less than 10% percent of baseline.

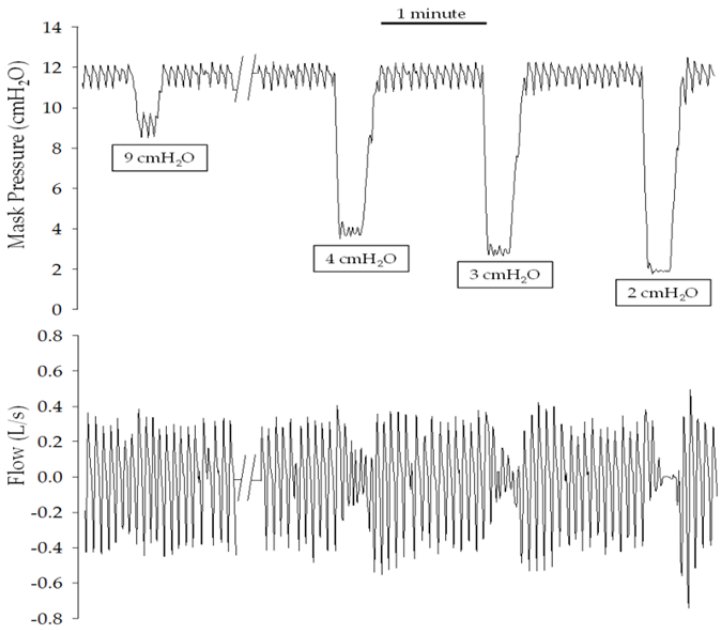


Figure 4. An illustration of the data collection procedures used to determine the critical closing pressure that demarcated collapse of the upper airway. Continuous positive airway pressure was decreased in a step wise fashion. The duration of each step was 3 to 5 breaths and was separated by the return to a holding pressure for 1 minute. Step wise decreases were administered until flow was abolished (i.e. apnea) and the corresponding pressure was ascertained.

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 (McGinley *et al.*, 2008a) characterized by consecutive 1-2 cmH_2O reductions in mask pressure, which were sustained for a duration of 5 minutes and were not separated by a recovery period at the holding pressure. We rationalized that establishing a holding pressure prior to a step-down in mask pressure lasting 3-5 breaths would be accompanied by upper airway muscle activity. Moreover, we considered that reductions in airway pressure are detected in a short period of time and result in the activation of

the upper airway muscles within 50 ms (Horner *et al.*, 1991;Horner *et al.*, 1994). 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. In order 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 (Figure 3 – 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 minutes (McGinley *et al.*, 2008a).

Instrumentation

During the 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 (Respitrace, 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 O_2 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. 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 polysomnography studies were analyzed for sleep stage, arousals, and respiratory-related events according to standard published criteria (Iber C *et al.*, 2007). 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 4th 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 minute period of stable non-rapid eye movement sleep prior to completing the protocol on visit 5 or 6. Baseline measures for 10 of the 13 participants were published previously (El-Chami *et al.*, 2014a). 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 prior to the initial step down (Figure 4). 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 (Figure 4). In order 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 minutes of the baseline holding period were analyzed. Thereafter, flow was measured from breaths recorded during the final 2 minutes of each pressure step down to determine the critical closing pressure. If participants aroused from sleep prior to determining the critical closing pressure during this visit, this measure was extrapolated using a second-order polynomial regression from the available pressure steps recorded prior to 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 to the average of the evening/afternoon was determined in order 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:

$$[y = m + a \cos (2\pi t/\omega) + \phi]$$

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), ω is the period of the circadian rhythm, and ϕ 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 analysis of variance on ranks combined with Student-Newman-Keuls post hoc test was used to compare event duration in participants that experienced either an increase or a decrease in event frequency during N2. A similar analysis was used to compare the partial pressure of end-tidal carbon dioxide in the evening, morning and afternoon. A two-way repeated measures analysis of variance 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 indices, 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 i) the critical closing pressure was correlated to the baseline apnea/hypopnea index ii) the average baseline measures of end-tidal carbon dioxide were correlated to the average critical closing pressure measured during the evening, morning and afternoon; iii) 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 ± S.E. A P value ≤ 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

Table 1. Baseline anthropometric, blood pressure and sleep measures

Variable	
Age, yr.	29.5 ± 1.9
Height, cm	174.5 ± 4.0
Weight, kg	82.6 ± 2.7
Body mass index, kg/m^2	26.4 ± 0.6
Systolic pressure, mmHg	119.2 ± 2.6
Diastolic pressure, mmHg	72.7 ± 2.8
Epworth Sleepiness Scale	10.0 ± 1.0
Apnea/hypopnea index, events/hr	41.8 ± 4.7
Lowest oxygen desaturation during apnea, %	87.2 ± 1.2
Race	7 AA, 4 Caucasian, 1 Asian, 1 Indian

Values are means ± SE

therapeutic pressure required to eliminate apnea during sleep on visit 3 in the OSA participants was 11.2 ± 0.8 cmH_2O. Average measures of core body temperature, recorded during visit 4 of the protocol, are shown in Figure 5. The nadir of temperature was evident in the early morning during visit 4 (Figure 5) and in the course of the visit used to measure the critical closing pressure.

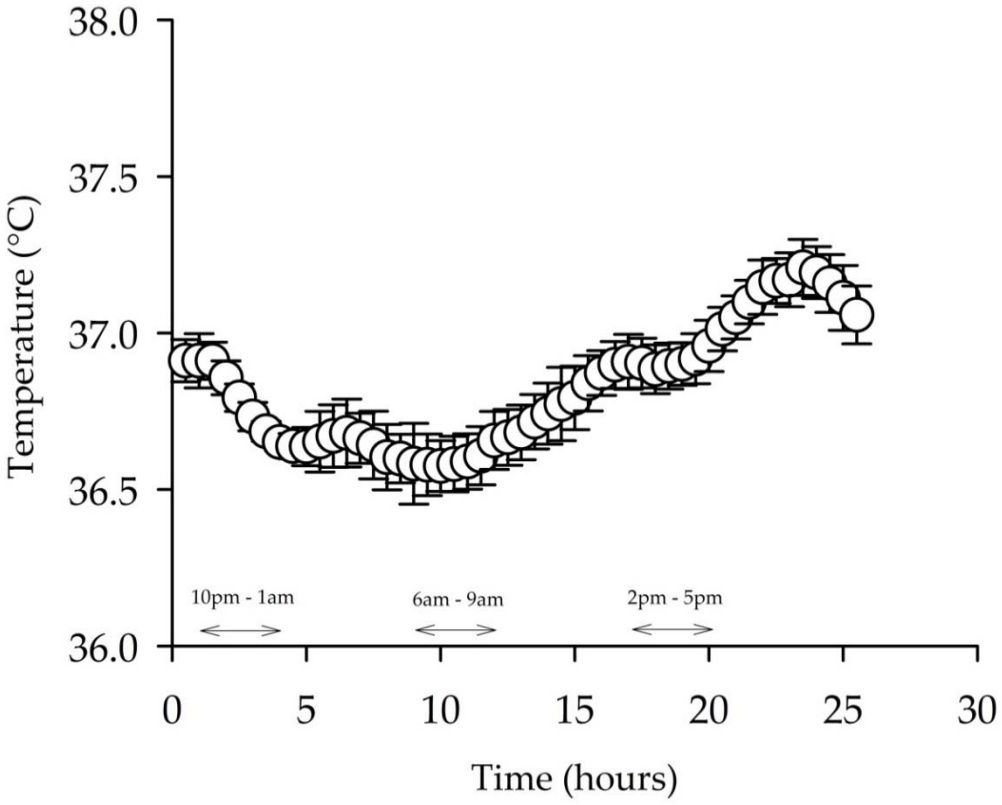


Figure 5. Average values of core body temperature in 12 participants with sleep apnea shown in 30 minute increments over a 24 hour cycle. Note that the nadir in temperature occurred at 6 am and the peak at 7:30 pm. Zero time represents the onset of the temperature recording (9:00 p.m.).

During visit 4, the participants spent 73.4 ± 4.9 %, 78.0 ± 2.7 % and 71.4 ± 3.6 %, of the total session time (i.e. 3 hours) in N1 and N2 of non-rapid eye movement sleep in the evening, morning and afternoon , respectively ($P \geq 0.47$). The percentage of time spent in N1 or N2 for a given 3-hour sleep session was not significantly different between the evening, morning and afternoon (Table 2) ($P \geq 0.85$). Figure 6A shows the average duration of breathing events during

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

	Evening	Morning	Afternoon
Wake (%)	24.7 ± 5.1	9.3 ± 2.3	19.9 ± 3.8
N1 (%)	22.7 ± 3.7	19.2 ± 2.8	20.3 ± 2.5
N2 (%)	47.6 ± 3.4	53.0 ± 4.0	49.0 ± 3.7
N3 (%)	3.1 ± 1.6	5.8 ± 2.5	2.1 ± 1.1
REM (%)	1.9 ± 0.9	12.6 ± 2.9	8.7 ± 2.8

Values are means ± SE. REM – Rapid Eye Movement Sleep.

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 to the afternoon and evening in N1 and N2 of non-rapid eye movement sleep ($P \leq 0.001$). In addition, the duration of breathing events was greater during N2 compared to N1 in the evening, morning and afternoon ($P \leq 0.001$).

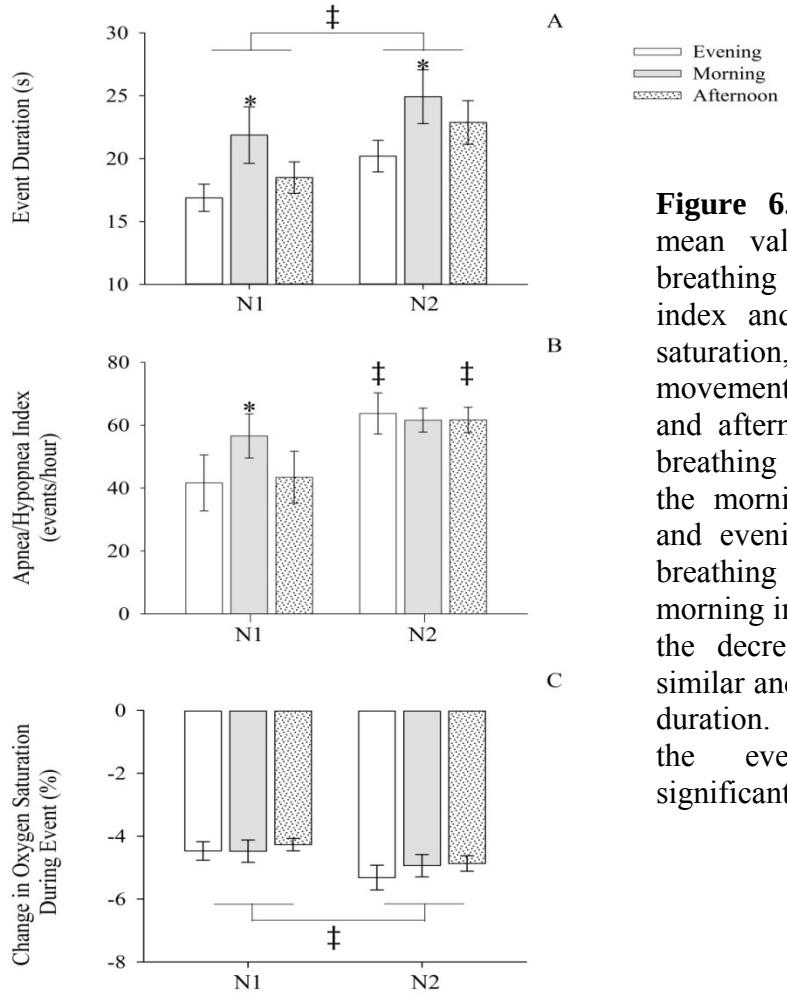


Figure 6. Histograms which show the mean values for (A) the duration of breathing events (B) the apnea hypopnea index and (C) the decrease in oxygen saturation, for N1 and N2 of non-rapid eye movement sleep in the evening, morning and afternoon sleep sessions. Note that breathing event frequency was greater in the morning compared to 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.

The apnea/hypopnea index was greater in the morning compared to the evening ($P \leq 0.01$) and afternoon ($P \leq 0.02$) in N1 of non-rapid eye movement sleep (Figure 6B). In contrast, the apnea/hypopnea index during the evening, morning and afternoon were similar across the 3 sleep sessions in N2 (Figure 6B). Further examination of the data revealed that an increase in event frequency was evident in 6 participants, while a decrease was evident in the remaining participants, in the morning compared to evening. The decrease in event frequency was coupled to a longer event duration compared to the values associated with increases in event frequency (30.3 ± 2.8 vs. 22.8 ± 3.3 s; $P \leq 0.04$).

The apnea/hypopnea index measured during N2 was greater compared to N1 during the evening ($P \leq 0.02$) and afternoon sleep sessions ($P \leq 0.04$). 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 to the afternoon and evening in N1 and N2 (Figure 6C). The decrease in oxygen saturation during breathing events in N2 was significantly greater compared to N1 independent of the time of day ($P \leq 0.002$).

The critical closing pressure using brief step-downs in pressure (i.e. 3-5 breaths) was more positive in the morning compared to the evening ($p \leq 0.02$) and afternoon ($P \leq 0.01$) (n=13) (Figure 7A). These relationships were unchanged ($P \leq 0.001$) when the critical closing pressure was determined using pressure step-downs of longer duration (n=8) (Figure 7B – right side). No difference in the critical closing pressure was evident in the evening, morning or afternoon when measures obtained from 8 participants using pressure step-downs of short (3 breaths) and long (5

minutes) duration were compared (Figure 7B). The average critical closing pressure was correlated to the baseline apnea/hypopnea index ($r = 0.60$, $p \leq 0.03$; $r = 0.74$, $p \leq 0.01$ with one outlier removed – see Figure 8A for additional details).

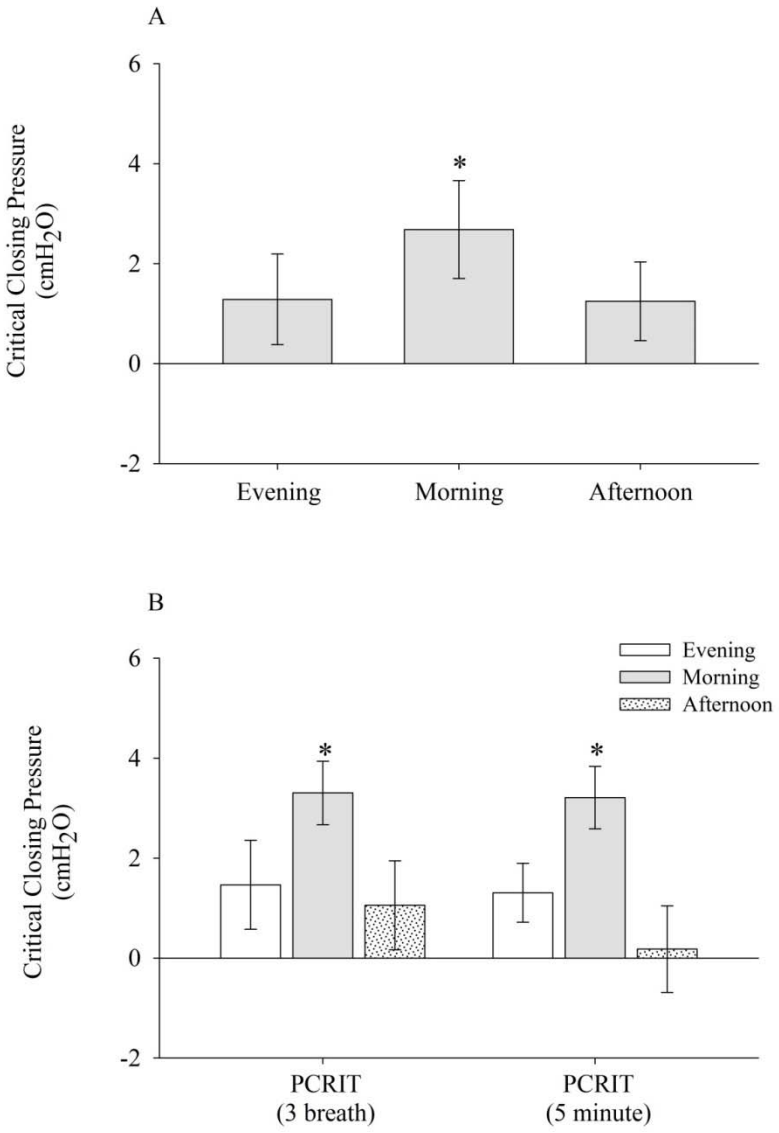


Figure 7. Histograms which show the mean critical closing pressure in the evening, morning and afternoon measured using (A) brief step-downs in pressure for 3-5 breaths (n=13) and (B) step-downs in pressure lasting 3-5 breaths and 5 minutes (n=8). Note that independent of the duration of each step-down in pressure, the critical closing pressure that demarcated the upper airway collapsibility was greater in the morning compared to the evening and afternoon. (*) – significantly different from the evening and afternoon. PCRIT – critical closing pressure.

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 to the evening and afternoon (El-Chami *et al.*, 2014a). This relationship remained unchanged with data from 3 additional participants included (evening 41.4 ± 0.6 vs. morning 40.2 ± 0.4 vs. afternoon 40.6 ± 0.5 mmHg; $P \leq 0.01$). 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 3 sleep sessions ($r = -0.59$, $P \leq 0.04$). The highest baseline levels of $P_{ET}CO_2$ 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, were strongly correlated to the change in critical closing pressure in the morning ($r = -0.73$, $P \leq 0.005$) (Figure 8B). The greater the decrease in baseline partial pressure of carbon dioxide the greater the increase in the critical closing pressure and vice versa.

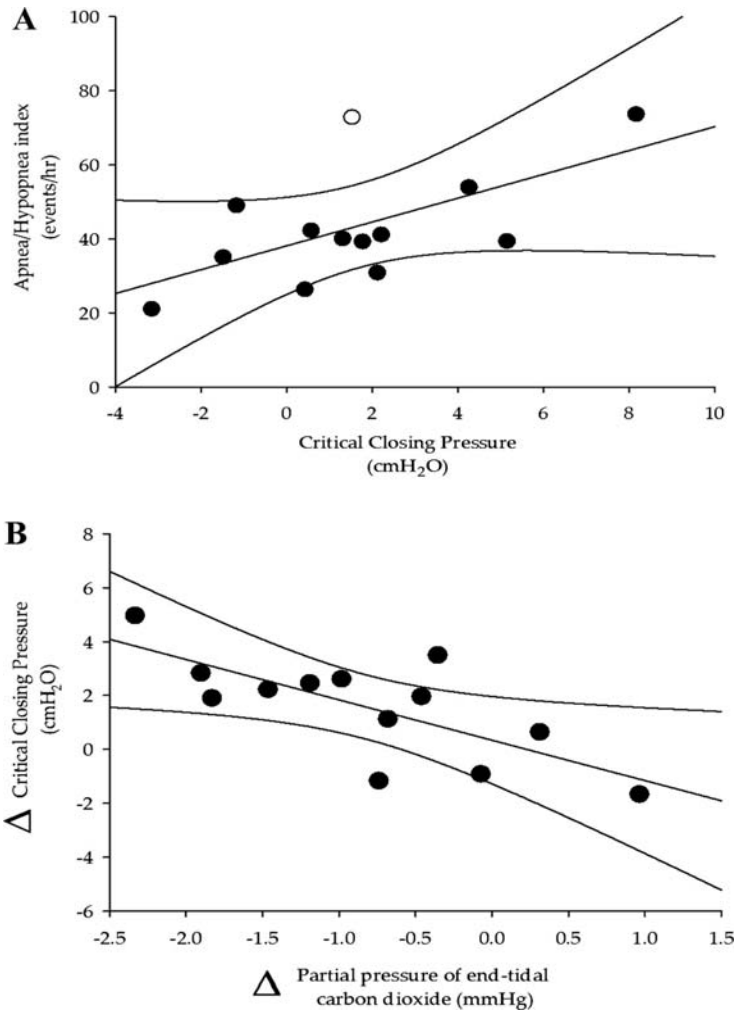


Figure 8. (A) A scatterplot which shows the relationship between the average critical closing pressure, measured in the morning, evening and afternoon for each participant, and the baseline apnea/hypopnea index. Note that the open circle represents a value that was significantly outside of the 99 % confidence intervals shown on the plot. (B) A scatterplot which shows the correlation between the change in carbon dioxide in the morning compared to measures in the evening and afternoon, and the change in the critical closing pressure in the morning compared to values measured in the evening and afternoon.

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 to 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 to the evening and afternoon. Secondarily, we also showed that the duration and frequency of breathing events was greater in N2 compared to 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 co-morbidities. 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 non-neuromuscular properties. Non-neuromuscular properties and neuromuscular properties that influence upper airway collapsibility may be separated if the airway is studied under conditions

of hypo and hypertonia, respectively (McGinley *et al.*, 2008a;Schwartz *et al.*, 1998). 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, prior to completing brief step-downs in pressure, we maintained a holding pressure that was 1-2 cmH_2O below the therapeutic pressure. This reduction in pressure was accompanied by a flow reduction of 10-15 %. 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 (Horner *et al.*, 1991;Horner *et al.*, 1994)] 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 (McGinley *et al.*, 2008a). We surmised that if the critical closing pressure using the brief step-downs in pressure was principally a reflection of non-neuromuscular influences on the upper airway, contrary to our postulation, then the critical closing measurements would be more positive compared to 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 which 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

(McGinley *et al.*, 2008b).

Arousal state effects on breathing event characteristics

A secondary finding from our investigation was that the frequency and duration of breathing events was greater during N2 compared to N1. These results are contrary to Ratnavadivel and colleagues (Ratnavadivel *et al.*, 2009) findings, which showed that the frequency of breathing events was reduced in N2 compared to N1. Ratnavadivel and colleagues (Ratnavadivel *et al.*, 2009) 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 to 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 re-establishment 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 (Eckert & Younes, 2014a). Although direct comparisons of sleep depth in N2 between studies is not possible, it is interesting to note that participants spent approximately 22 % of non-rapid eye movement sleep in slow wave sleep in Ratnavadivel and colleagues investigation (Ratnavadivel *et al.*, 2009) 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 to the first half of the night, independent of sleep stage (Cala *et al.*, 1996;Charbonneau *et al.*, 1994;Fanfulla *et al.*, 1997;Lavie *et al.*, 1981;Montserrat *et al.*, 1996a;Sforza *et al.*, 1998). 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 to measures obtained during sleep in the evening, but also compared to 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 (Cala *et al.*, 1996;Charbonneau *et al.*, 1994;Lavie *et al.*, 1981; Montserrat *et al.*, 1996a;Sforza *et al.*, 1998). 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 (Charbonneau *et al.*, 1994;Fanfulla *et al.*, 1997;Sforza *et al.*, 1998). Consequently, increases in event frequency or duration during the second compared to 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 (Cala *et al.*, 1996;Lavie *et al.*, 1981;Montserrat *et al.*, 1996a). 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 to the evening and afternoon in N1 but not in N2. Alternatively, the duration of breathing events increased in the morning compared to 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 to 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, was significantly less in the morning compared to the evening and afternoon (El-Chami *et al.*, 2014a). The addition of 3 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 to 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 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 to the morning [see (Zhang *et al.*, 2009) for review]. These results have been obtained in a variety of muscles ranging from the adductor pollicis (Martin *et al.*, 1999) to leg extensor muscles (Sedliak *et al.*, 2008). Likewise, a decrease in the rate of tension development and one-half relaxation time has been reported in the morning compared to the evening and afternoon (Martin *et al.*, 1999).

Mechanisms responsible for the circadian modulation of upper airway collapsibility

There are multiple inputs and stimuli (Figure 9) 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 i) blunted feedback from upper airway receptors in response to inflammation, edema and/or neural damage (Cala *et al.*, 1996) or ii) enhanced chemoreceptor feedback in response to intermittent hypoxia (Mateika & Narwani, 2009;Mateika & Syed, 2013a;Mateika *et al.*, 2015;Syed *et al.*, 2013;Yokhana *et al.*, 2012). 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 to the afternoon and evening. Serotonin, which is a neuromodulator of hypoglossal motor neuron activity, varies in a diurnal pattern (Agren *et al.*, 1986;Mateos *et al.*, 2009;Sun *et al.*, 2002). Likewise, Volgin and colleagues (Volgin *et al.*, 2013) 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 serotoninergic neurons in the raphe pallidus and obscurus project to hypoglossal motor neurons (Manaker & Tischler, 1993). These serotoninergic neurons sense carbon dioxide levels (Richerson, 2004), which increase genioglossus muscle activity in healthy humans during wakefulness (Onal *et al.*, 1981;Patrick *et al.*, 1982), and healthy humans (McSharry *et al.*, 2013;Saboisky *et al.*, 2010a) or humans with obstructive sleep apnea (Loewen *et al.*, 2011a) 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 to the evening and afternoon coincident with the nadir in core body temperature and $P_{ET}CO_2$ (El-Chami *et al.*, 2014a). Coincident reductions in $P_{ET}CO_2$ 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 (Figure 9) could also elicit fluctuations in carbon dioxide that promote increased upper

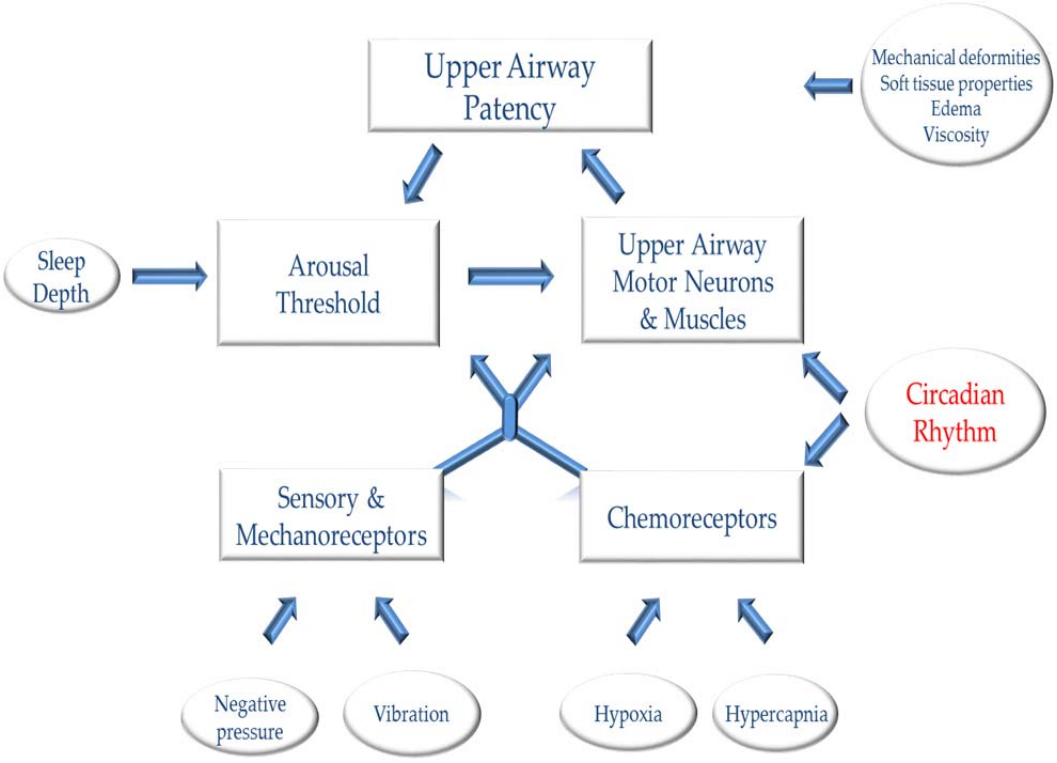


Figure 9. A schematic diagram showing a variety of inputs that impact on upper airway collapsibility. Note that a number of stimuli (e.g. intermittent hypoxia, upper airway vibration etc.) that progressively increase throughout the night as a consequence of sleep apnea activate a variety of afferents that affect upper airway motor neuron and muscle activity. These inputs could account for time of day effects on the duration and frequency of breathing events. However, note that an endogenous circadian modulation of upper airway collapsibility, which may be mediated by direct inputs to upper airway motor neurons and indirectly via adjustments in chemoreflex sensitivity, occur despite the elimination of sleep apnea.

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 to the evening and afternoon (El-Chami *et al.*, 2014a). 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 to the afternoon and evening. Likewise, given that the response to hypoxia is blunted or absent in the presence of hypocapnia (Rapanos & Duffin, 1997a;Wilson & Day, 2013a;Moore *et al.*, 1984;Weil *et al.*, 1970a), it is possible that hypoxia does not significantly enhance receptor feedback prior to 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 to the first half of the night in individuals with sleep apnea has 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 (Cala *et al.*, 1996). 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 to 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 complexion of breathing events across the night (Deacon & Catcheside, 2014;Mahamed *et al.*, 2003;Mateika, 2015). In addition to these possibilities, our published findings (El-Chami *et al.*, 2014a) 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.

CHAPTER 3 - THE EFFECT OF TIME OF DAY ON CHEMOREFLEX SENSITIVITY AND THE CARBON DIOXIDE RESERVE DURING NREM SLEEP IN PARTICIPANTS WITH SLEEP APNEA

(This chapter contains previously published material. See Appendix C)

Introduction

Clinical studies have reported that the number (Fanfulla *et al.,* 1997; Salloum *et al.*, 2010) and duration of breathing events increase throughout the night (Charbonneau *et al.,* 1994; Fanfulla *et al.,* 1997; Salloum *et al.*, 2010) and that this increase is independent of sleep architecture and body posture. Likewise, a reduction in apneic events during daytime compared to nighttime sleep has been predicted by computer modeling simulations (Spengler *et al.* 2000), and supported by experimental evidence which showed that the apnea-hypopnea index was reduced during sleep in the day compared to the night in a small number of hypertensive men (Robin *et al*, 1958).

The potential mechanisms underlying the increase in apnea severity are likely multifactorial and phenotypically dependent (Dempsey *et al.*, 2010;Orr *et al.*, 2014;Younes, 2014). One possibility is that increases in chemoreflex sensitivity, coupled to a reduction in the carbon dioxide reserve, are responsible for the progressive increase in breathing events across the night (Mateika & Narwani, 2009;Mateika & Syed, 2013a). This suggestion is supported indirectly by Mahamed and colleagues, who reported that chemoreflex sensitivity to hypercapnia/hyperoxia increased during wakefulness in the morning, following six hours of sleep, compared to the evening in sleep apnea participants (Mahamed *et al.*, 2005). Sforza and colleagues 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 in oxygen desaturation during apneic events were constant (Sforza *et al.*, 1998). These findings, along with the established understanding that

enhanced chemoreflex sensitivity promotes the occurrence of both central and obstructive breathing events provides support for the possibility that alterations in chemoreflex properties contribute to the promotion of breathing events at different points throughout the sleep period (Dempsey *et al.*, 2004;Dempsey, 2005;Dempsey *et al.*, 2010;Mateika & Narwani, 2009;Mateika & Syed, 2013a;Yokhana *et al.*, 2012).

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 reported increase in chemoreflex sensitivity during wakefulness in the morning compared to the evening in patients with sleep apnea (Mateika & Narwani, 2009;Mateika & Syed, 2013a). 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 (Griffin *et al.*, 2012;Mateika *et al.*, 2004a;Morelli *et al.*, 2004;Wadhwa *et al.*, 2008) and individuals with sleep apnea (Gerst, III *et al.*, 2011;Khodadadeh *et al.*, 2006). The second possibility is that alterations in chemoreflex properties reflect an endogenous oscillation that is linked to time of day. Indeed Spengler et al. (Spengler *et al.*, 2000) and Stephenson et al. (Stephenson *et al.*, 2000) have reported that chemoreflex sensitivity during wakefulness in healthy participants is modulated by a circadian rhythm. Despite these findings, no studies have examined in individuals with sleep apnea if chemoreflex properties (i.e. apneic threshold, carbon dioxide reserve and chemoreflex sensitivity) 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 to 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 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 that completed the protocol visited the laboratory on 6 occasions. During the first visit to the laboratory, written informed consent was obtained and thereafter a physical examination, health and lifestyle questionnaires, blood pressure and lung volume measures along with a 12-lead EKG 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 Inc., Murraysville, PA). The watch was used to monitor the sleep-wake schedule of the participants while they slept at home for two weeks prior to 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 two week 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 prior to obtaining

formal measurements.

After the two week time 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 for a minimum of 27 hours. 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 prior to 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. Upon arrival the participants ingested a radiotelemetry pellet (CorTemp® Sensor, HQInc, Palmetto, FL) in order 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 a minimum time period of 27 hours. Following instrumentation, participants slept for three hours during 3 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 minutes and immediately thereafter read for 90 minutes. Ninety minutes prior to 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 minutes composed of approximately 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 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 (Figure 3A). Mechanical ventilation was applied for 3 minutes in a spontaneous-timed pressure support mode, as described in previous publications (Mateika *et al.*, 2004b;Salloum *et al.*, 2010). In this mode, a backup respiratory rate of 6–8 breaths/min was preset and timed breaths delivered if the participant's respiratory rate fell below the set rate. The ventilator respiratory rate, which was below the participant's eupneic rate, was set to prevent neuromechanical inhibition of ventilatory motor output. During mechanical ventilation, the inspiratory positive airway pressure was increased gradually in 1 to 2 cmH_2O 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 over distension of the airway. Mechanical ventilation was terminated after 3 minutes, 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 (Figure 10A). 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 expiratory time $\geq$ 5 s. Each hyperventilation trial was separated by a five minute period of baseline breathing.

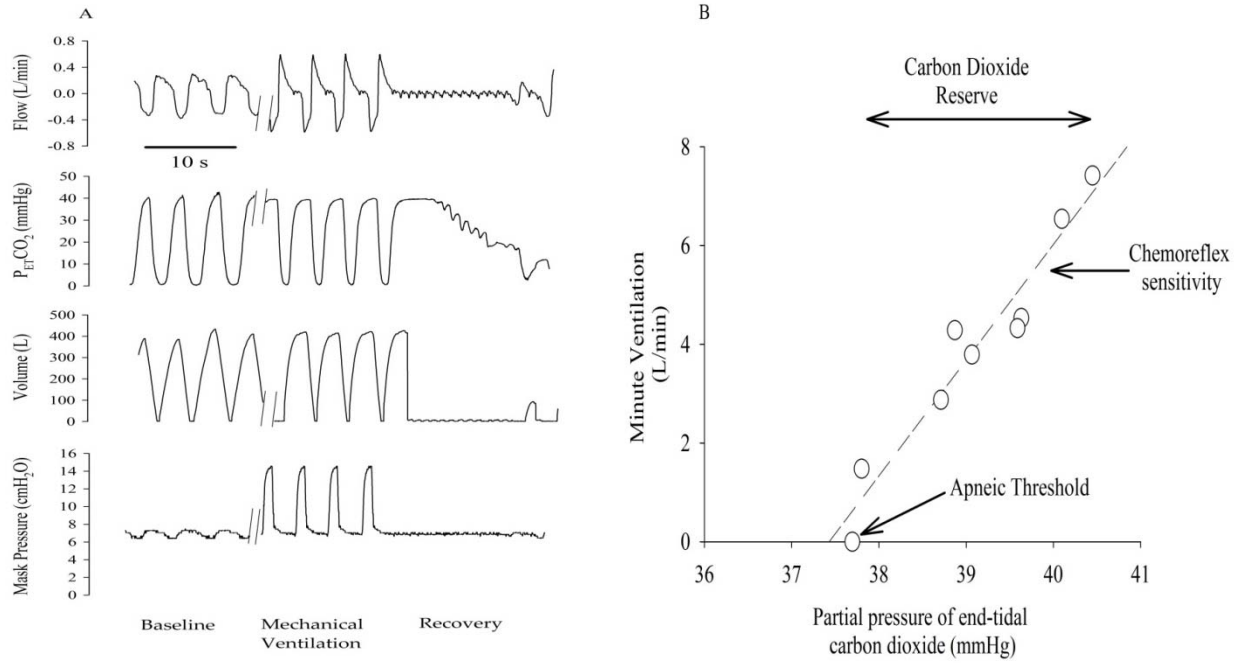


Figure 10. An illustration of the data collection and analysis procedures used to elicit a hypocapnic induced apnea. (A) **A** single trial which shows that after measuring baseline ventilation and the partial pressure of carbon dioxide ($P_{ET}CO_2$), mechanical hyperventilation reduced carbon dioxide 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 step-wise reductions in $P_{ET}CO_2$. In this example, reductions in $P_{ET}CO_2$ occurred over 9 separate trials (open circles) until an apnea was achieved. The difference between baseline $P_{ET}CO_2$ and the $P_{ET}CO_2$ 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.

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 (Respitrace, Ambulatory monitoring, Inc., Ardsley, NY). Airflow and breath timing (inspiratory and expiratory time) were measured using a pneumotachometer (Model RSS100-HR, Hans Rudolph Inc., Shawnee, KS) attached to a nasal mask. Oxygen saturation (SaO_2) 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 Inc., Ventura, CA) were obtained from air expired into sampling tubes attached to ports on the face 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, Inc., Houston, TX) to confirm apnea and ascertain the presence of flow limitation. All physiological variables were analogue 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 Inc., West Warwick, RI). The cardio-respiratory variables were also input into a second computer using a commercially available software package (WinDaq, Dataq Instruments Inc., 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 two minutes that immediately preceded the onset of mechanical ventilation. An average of the baseline periods measured during a given sleep session was calculated. The average of 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 $P_{ET}CO_2$ was calculated as the difference between the $P_{ET}CO_2$ recorded during the control period and the $P_{ET}CO_2$ associated with the last five mechanically ventilated breaths. Minute ventilation was given a value of 0 once a central apnea was induced.

The apneic threshold was defined by the $P_{ET}CO_2$ associated with the occurrence of an apnea (Figure 10B). The carbon dioxide reserve was defined as the difference in $P_{ET}CO_2$ measured during baseline breathing and the $P_{ET}CO_2$ that demarcated the occurrence of an apnea (Figure 10B). Chemoreflex sensitivity was determined by dividing the change in minute ventilation (i.e. the difference between minute ventilation measured during baseline and minute ventilation measured once the ventilator was returned to an expiratory positive airway pressure) and the change in $P_{ET}CO_2$ (i.e. the difference between $P_{ET}CO_2$ measured during baseline and the $P_{ET}CO_2$ that demarcated an apnea) (Figure 10B).

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 ($y = m + a\cos(2\pi t/\omega+\phi)$; 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), ω is the period of the circadian rhythm, ϕ is the phase angle. Once the phase and period of the core body temperature was 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 was 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 $P_{ET}CO_2$ and minute ventilation, the $P_{ET}CO_2$ 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 3 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/hypopnea index ranged from mild to severe according to standard criteria. However, 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 while scores from the Stanford sleepiness scale indicated normal alertness for both groups on the day of the screening visit (Table 3). The average therapeutic pressure required to eliminate apnea during sleep on visit 2 in the OSA participants was 11.2 ± 0.7 cmH_2O.

Table 3. Baseline anthropometric, blood pressure and sleep measures

Variable	
Age, yr.	29.8 ± 2.1
Height, cm	172.5 ± 5.0
Weight, kg	80.7 ± 3.2
Body mass index, kg/m^2	26.2 ± 0.7
Systolic pressure, mmHg	121.7 ± 2.0
Diastolic pressure, mmHg	74.2 ± 2.9
Epworth Sleepiness Scale	10.3 ± 1.3
Apnea/hypopnea index, events/hr	39.1 ± 5.1
Lowest oxygen desaturation during apnea, %	87.5 ± 1.1
Race	6 AA, 2 Caucasian, 1 Asian, 1 Indian

The nadir of core body temperature was evident during the morning session (Figure 11). Baseline measures of minute ventilation were lower in the morning compared to values in the evening ($p < 0.02$) and afternoon ($p < 0.02$) (Figure 12A). A corresponding decrease in tidal volume (Figure 12B), but not breathing frequency (Figure 12C), was evident in the morning compared to the evening ($p < 0.04$) and afternoon ($p < 0.04$). Baseline $P_{ET}CO_2$ was reduced in the morning ($p < 0.002$) and afternoon ($p < 0.02$) compared to the evening (Figure 13); while the

$P_{ET}CO_2$ values that demarcated the apneic threshold were similar across the evening, morning and afternoon sessions (Figure 13). The carbon dioxide reserve was reduced in the morning (2.1 ± 0.25 mmHg) compared to the evening (3.6 ± 0.5 mmHg) ($p < 0.002$) and afternoon (3.5 ± 0.3 mmHg) ($p < 0.001$) (Figure 13), while chemoreflex sensitivity was increased in the morning compared to the evening ($p < 0.001$) and afternoon ($p < 0.001$) (Figure 12D).

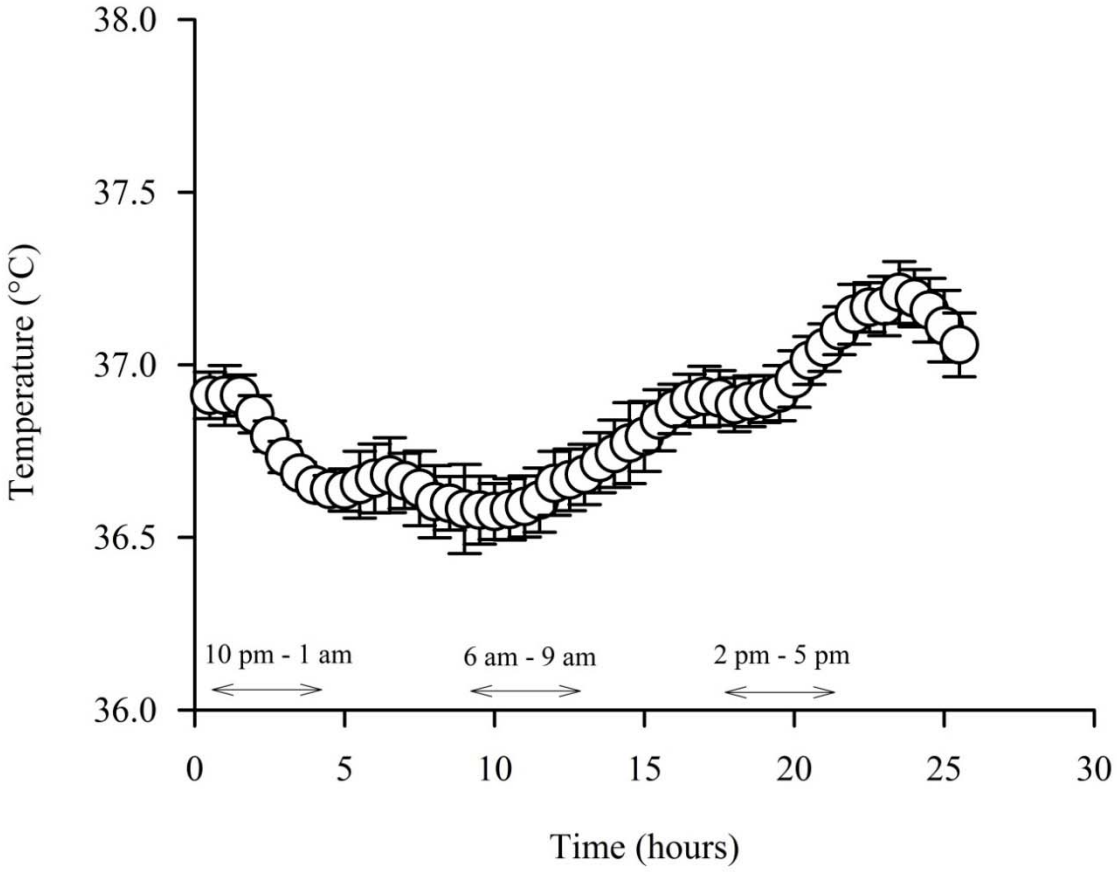


Figure 11. Average values of core body temperature of 10 participants with sleep apnea shown in 30 minute increments over a 24 hour cycle. Note that the nadir in temperature occurred at 6 am and the peak at 7:30 pm (vertical arrows). Zero time represents the onset of the temperature recording (9:00 p.m.).

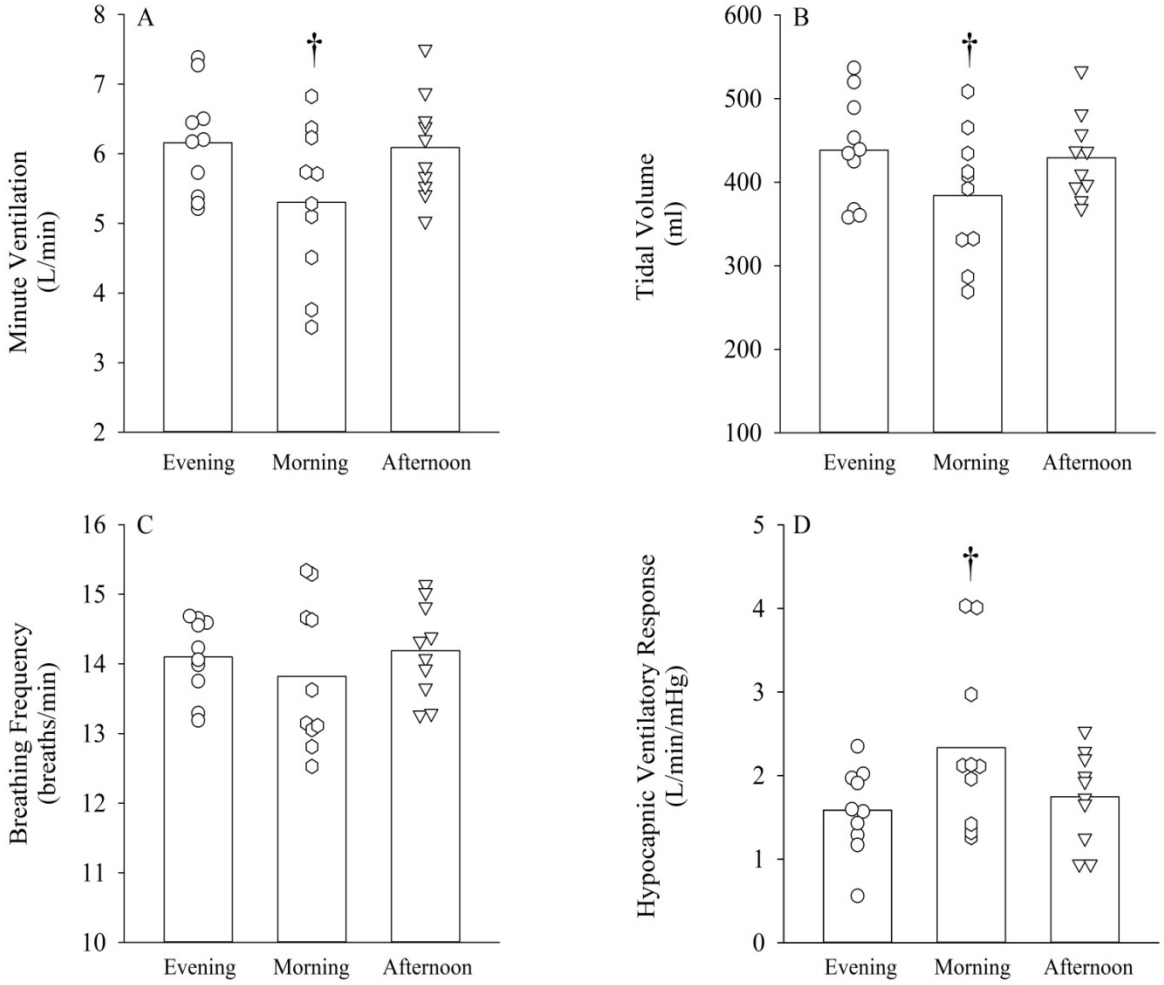


Figure 12. Combined histograms and scatterplots which show the group mean, and mean values for each participant, calculated from measures of (A) baseline minute ventilation (B) baseline tidal volume (C) baseline breathing frequency and (D) the hypocapnic ventilatory response, during the evening, morning and afternoon sleep sessions. Note that minute ventilation along with tidal volume was reduced and the hypocapnic ventilatory response increased in the morning compared to the afternoon and evening. † significantly different compared to the evening and afternoon.

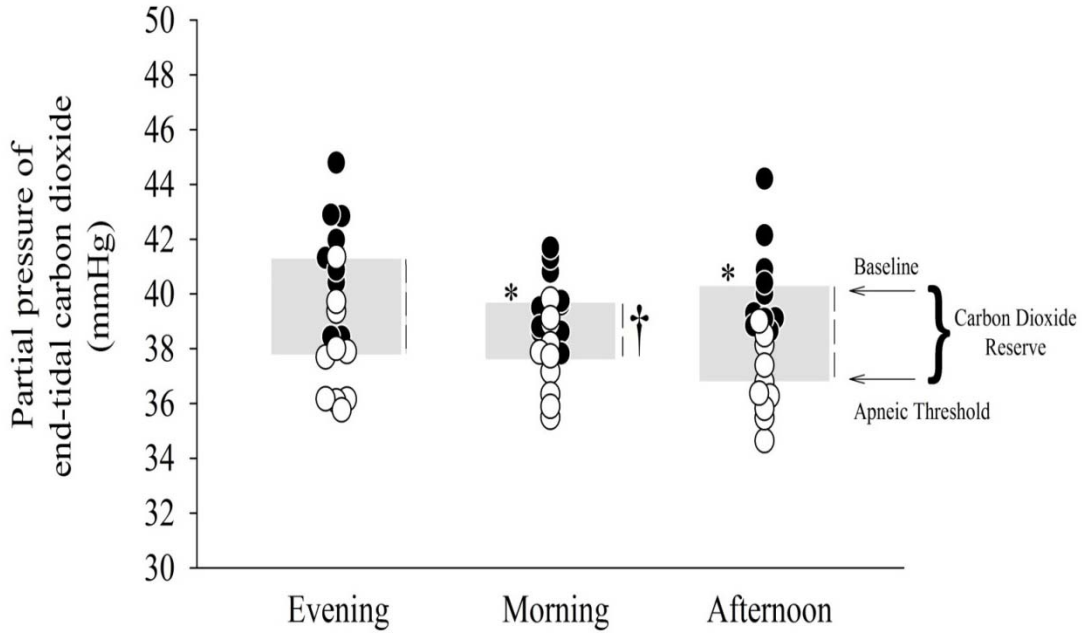


Figure 13. Combined histograms and scatterplots which show the group mean, and mean values for each participant, calculated from baseline measures of the partial pressure of carbon dioxide ($P_{ET}CO_2$) (group average indicated by top of the gray bars and individual data indicated by the black circles), the $P_{ET}CO_2$ that demarcates the apneic threshold (group average indicated by bottom of the gray bars and individual data indicated by white circles) and the carbon dioxide reserve indicated by the vertical dashed line parallel to each histogram bar. Note that baseline $P_{ET}CO_2$ was reduced in the morning and afternoon compared to the evening (*) and that the carbon dioxide reserve was reduced in the morning compared to the evening and afternoon (†).

Discussion

We employed a constant routine protocol to measure chemoreflex properties during non-rapid eye movement sleep across the 24-hour 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 to the evening and afternoon.

Baseline measures of ventilation $P_{ET}CO_2$, temperature and time of day

Previous studies have employed constant routine protocols to directly measure ventilation over a 24 hour period of wakefulness in healthy humans breathing room air (Adamczyk *et al.*, 2008;Spengler *et al.*, 2000;Vargas *et al.*, 2001), or breathing air comprised of elevated levels of carbon dioxide (Stephenson *et al.*, 2000) or reduced levels of oxygen (Vargas *et al.*, 2001).

Collectively, a circadian oscillation in minute ventilation (Adamczyk *et al.*, 2008;Spengler *et al.*, 2000;Stephenson *et al.*, 2000), or it components tidal volume and breathing frequency (Adamczyk *et al.*, 2008), has been reported; although the circadian rhythm of minute ventilation approached but did not reach statistical significance in one investigation (Spengler *et al.*, 2000). The nadir of minute ventilation occurred in the early morning in the majority of studies (Adamczyk *et al.*, 2008;Stephenson *et al.*, 2000), but in one case was evident in the early evening (Spengler *et al.*, 2000). Our results are similar to published findings since a significant reduction in minute ventilation and tidal volume was evident in the morning compared to 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 (Mortola, 2004). However, our results, coupled with previous findings (Adamczyk *et al.*, 2008;Spengler *et al.*, 2000;Stephenson *et al.*, 2000), 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 (Adamczyk *et al.*, 2008;Spengler *et al.*, 2000;Stephenson *et al.*, 2000) and the present investigation (e.g. 24 hours of sleep deprivation vs. 3 hour 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 $P_{ET}CO_2$ over a 24 hour period of wakefulness, while

breathing room air, has also been measured in a few studies (Spengler *et al.*, 2000;Stephenson *et al.*, 2000;Vargas *et al.*, 2001). In these studies a well-established oscillation in core body temperature (Hofstra & de Weerd, 2008) was evident with the nadir occurring during the early morning hours (i.e. 6 - 8 am) (Spengler *et al.*, 2000;Stephenson *et al.*, 2000;Vargas *et al.*, 2001). However, the relationship between minute ventilation and core body temperature varied, with core body temperature in phase with minute ventilation in one investigation (Stephenson *et al.*, 2000) but lagging 6-8 hours in another (Spengler *et al.*, 2000).

An obvious but small oscillation (~ 1-2 mmHg) in $P_{ET}CO_2$ has also been documented (MILLS, 1953;Spengler & Shea, 2000;Vargas *et al.*, 2001), and the relationship between minute ventilation and $P_{ET}CO_2$ was reportedly in phase (i.e. decreases in ventilation were accompanied by decreases in $P_{ET}CO_2$) with the nadir of the measures occurring between 6 pm and midnight (Spengler & Shea, 2000). 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 $P_{ET}CO_2$ were significantly lower during sleep in the morning compared to 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 definitely that minute ventilation and $P_{ET}CO_2$ were in phase with the nadir of core body temperature. However, given our results it is unlikely that the nadir of core body temperature lagged minute ventilation and $P_{ET}CO_2$ to the degree reported in a previous investigation (Spengler & Shea, 2000).

It has long been established that $P_{ET}CO_2$ is influenced by arousal state, increasing during sleep compared to wakefulness (Mortola & Maskrey, 2011;ROBIN *et al.*, 1958). However, our results, along with previous findings (MILLS, 1953;Spengler *et al.*, 2000;Vargas *et al.*, 2001), confirm that within a sleep or wake state $P_{ET}CO_2$ is effected by the time of day. Moreover, our results reveal that a coincident reduction in minute ventilation and $P_{ET}CO_2$ occurred in

conjunction with core body temperature. The mechanism responsible for the coincidental nadir in minute ventilation and $P_{ET}CO_2$ during the morning or the increase in minute ventilation and $P_{ET}CO_2$ in the other sessions is unknown. However, given the increase in chemoreflex sensitivity (see *Chemoreflex properties and time of day* for further discussion) that we measured in the morning, compared to the afternoon and evening, it seems unlikely that chemoreflex inputs were principally responsible for the coincidental decrease in ventilation and $P_{ET}CO_2$ 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 (Phillipson EA & Bowes G, 1986). Alternatively, given the coincident variations in core body temperature, minute ventilation and $P_{ET}CO_2$ in the present investigation, coupled with similar published findings that included measures of metabolic rate during wakefulness (Spengler *et al.*, 2000), indicates a robust link between minute ventilation and metabolism. Despite this result the mechanism responsible for the link between metabolism and minute ventilation remains unknown. Perhaps parallel inputs to the respiratory controller and temperature regulation network is responsible for the coincident reduction in minute ventilation, $P_{ET}CO_2$ and temperature that was observed. Indeed, activation of the nucleus tractus solitarius and ventrolateral medulla in rats induces a coincident decrease in temperature and $P_{ET}CO_2$ (Cao *et al.*, 2010).

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 (Fuse *et al.*, 1999;Mahamed *et al.*, 2005;Raschke & Moller, 1989;Siekierka *et al.*, 2007;Spengler *et al.*, 2000;Stephenson *et al.*, 2000). Some studies were designed to measure the threshold and/or chemoreflex sensitivity in the evening, prior to sleep, and in the morning, immediately after sleep (Fuse *et al.*, 1999;Mahamed *et al.*, 2005). The

results were variable in that the ventilatory response to increases in carbon dioxide during wakefulness were reported to be unchanged (Fuse *et al.*, 1999) or altered as a consequence of a decrease in the chemoreflex threshold (Mahamed *et al.*, 2005) in the morning compared to 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 (Fuse *et al.*, 1999;Mahamed *et al.*, 2005). Nonetheless, despite this variability the reported change (Mahamed *et al.*, 2005) or lack thereof (Fuse *et al.*, 1999) 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 hour period (Raschke & Moller, 1989;Siekierka *et al.*, 2007;Spengler *et al.*, 2000;Stephenson *et al.*, 2000). In two studies the participants were awake and inactive throughout the constant routine protocol (Spengler *et al.*, 2000;Stephenson *et al.*, 2000). In the remaining studies measurements were also made during wakefulness (Raschke & Moller, 1989;Siekierka *et al.*, 2007); however participants were either allowed to sleep at will (Raschke & Moller, 1989), or sleep for 2 hours from 4-6 a.m. in addition to engaging in moderate physical activity (Siekierka *et al.*, 2007). Results from these studies revealed that a circadian oscillation in the ventilatory response to isocapnic hypoxia (Raschke & Moller, 1989;Siekierka *et al.*, 2007) or the ventilatory response to carbon dioxide in the presence of normoxia (Raschke & Moller, 1989;Spengler *et al.*, 2000) or hypoxia (Stephenson *et al.*, 2000) 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 hour cycle (Siekierka *et al.*, 2007). Inclusion of exercise and one period of sleep in the constant routine protocol may have influenced the results (Siekierka *et al.*, 2007). Spengler and Shea 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 a.m.

and 2 p.m., while the bathyphase which was evident starting at 6 pm remained stable for 12-14 hours (Spengler *et al.*, 2000). In other studies the acrophase of the ventilatory response to carbon dioxide, either as a consequence of an increase in the chemoreflex threshold (Stephenson *et al.*, 2000) or a decrease in chemoreflex sensitivity (Raschke & Moller, 1989), was evident later in the evening (6 p.m.) while the onset of the bathyphase was manifested in the morning (Raschke & Moller, 1989;Stephenson *et al.*, 2000). 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 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 (Fuse *et al.*, 1999;Mahamed *et al.*, 2005). In both studies measures of the chemoreflex threshold (Mahamed *et al.*, 2005) and/or chemoreflex sensitivity (Fuse *et al.*, 1999;Mahamed *et al.*, 2005) were obtained during wakefulness in the evening, prior to sleep, and in the morning, immediately after sleep (Fuse *et al.*, 1999;Mahamed *et al.*, 2005). Mahamed and colleagues reported that the chemoreflex sensitivity to hyperoxia/hypercapnia was increased in the morning compared to the evening (Mahamed *et al.*, 2005), while Fuse reported that no changes in chemoreflex sensitivity were evident (Fuse *et al.*, 1999). Mahamed and colleagues (Mahamed *et al.*, 2005) suggested that the reasons for the discrepant findings was that Fuse et al. (Fuse *et al.*, 1999) used a standard hyperoxic rebreathing technique that measures ventilatory responses in the hypercapnic range of 50–80 mmHg, whereas Mahamed at al. used a modified rebreathing technique that measures ventilation in the normocapnic range of 35–60 mmHg (Mahamed *et al.*, 2005). 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 so that 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 (Fuse *et al.*, 1999;Mahamed *et al.*, 2005). 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 to the evening and afternoon. Conversely, the $P_{ET}CO_2$ that demarcated the apneic threshold remained stable across the 3 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 to 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 (Fanfulla *et al.*, 1997;Sforza *et al.*, 1998) and duration (Cala *et al.*, 1996;Charbonneau *et al.*, 1994;Sforza *et al.*, 1998) of breathing events increase throughout the night independent of sleep architecture and body posture. Likewise, a reduction in apneic events during daytime compared to nighttime sleep has been predicted by computer modeling simulations (Stephenson, 2004), and supported by experimental evidence which showed that the apnea-hypopnea index was reduced during sleep in the day compared to the night in a small number of hypertensive men (Scharf *et al.*, 1990).

The duration of breathing events might be increased as a consequence of diminished feedback from upper airway sensory receptors which Cala and colleagues have suggested is due to repeated trauma to pharyngeal tissues as a consequence of upper airway vibration and closure (Cala *et al.*, 1996). Alternatively, 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 (Mateika & Narwani, 2009;Mateika & Syed, 2013a). Indeed our findings support this suggestion, since chemoreflex sensitivity was increased and the carbon dioxide reserved decreased in the morning compared to the afternoon and evening.

One of the stimuli which could initiate increases in chemoreflex sensitivity is intermittent hypoxia, a hallmark of sleep apnea (Mateika & Narwani, 2009;Mateika & Syed, 2013a). Indeed numerous studies reported that exposure to intermittent hypoxia during wakefulness increases the ventilatory response to carbon dioxide and sustained hypoxia (Gerst, III *et al.*, 2011;Khodadadeh *et al.*, 2006;Mateika *et al.*, 2004a;Morelli *et al.*, 2004;Wadhwa *et al.*, 2008). 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 reserved is decreased in the morning compared to 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 effect of time of day on the administered dose of pharmaceuticals agents targeted toward mitigating the impact that increases in chemoreflex sensitivity and/or decreases in the carbon dioxide reserve have on breathing instability in patients with sleep apnea should be considered (Wang *et al.*, 2013).

CHAPTER 4 - IMPACT OF AROUSAL THRESHOLD AND CHEMOREFLEX SENSITIVITY ON APNEA FREQUENCY AND DURATION IN PARTICIPANTS WITH SLEEP APNEA

Introduction

We reported that the frequency and duration of apneic events is dependent on sleep stage (El-Chami *et al.*, 2015). More specifically, we showed that the duration and frequency of events was greater in N2 compared to N1 sleep, independent of the time of day (El-Chami *et al.*, 2015). The observed increase in event duration in N2 could be caused by an increase in the arousal threshold, along with an increase in the effective recruitment threshold for upper airway muscle activity (Cala *et al.*, 1996;Eckert & Younes, 2014b;Montserrat *et al.*, 1996b). Alternatively, a blunted respiratory response to afferent inputs (e.g. chemoreflex and mechanoreceptor inputs), reflected by a decrease in the rate of change of respiratory effort during apneic events in N2 could be responsible for sleep stage related differences in apneic duration. The relationship between event duration and arousal threshold or respiratory response sensitivity should impact on respiratory effort immediately prior to termination of an event, and closely after establishment of airway patency.

Kimoff and colleagues reported that the rate of change of respiratory effort during breathing events in N2 is independent of event duration (Cala *et al.*, 1996;Montserrat *et al.*, 1996b). Thus, respiratory effort at the termination of an event might reflect increases in the arousal or effective recruitment threshold. If these findings are consistent across sleep states the rate of change of respiratory effort during events in N1 compared to N2 may be similar. However, the maximum response at the termination of an event and immediately upon re-establishment of upper airway patency may be increased if the arousal threshold or effective recruitment threshold is increased in N2 compared to N1. The present investigation was completed in part to test this hypothesis.

In addition to the variation in event duration and frequency in N2 compared to N1, we also recently reported that event duration was greater in the morning compared to the afternoon and evening within a given sleep stage (i.e. N2) (El-Chami *et al.*, 2015), consistent with previous published findings (Cala *et al.*, 1996;Charbonneau *et al.*, 1994;Fanfulla *et al.*, 1997;Lavie *et al.*, 1981;Montserrat *et al.*, 1996a;Sforza *et al.*, 1998). Likewise, event frequency was greater in the morning during N1 (El-Chami *et al.*, 2015). We provided evidence which suggested that the effect of time of day on the duration and frequency of breathing within a given stage of sleep might be mediated by a circadian variation in both chemoreflex sensitivity (El-Chami *et al.*, 2014b), and upper airway collapsibility (El-Chami *et al.*, 2015). Explicitly, we found that an increase in chemoreflex sensitivity coupled to a decrease in the carbon dioxide reserve, and a more collapsible airway, was evident in the morning during N2 compared to the afternoon and evening (El-Chami *et al.*, 2014b). These findings were evident despite the absence of hallmarks of sleep apnea (i.e. intermittent hypoxia) (El-Chami *et al.*, 2014b).

The variation in chemoreflex properties could result in an increased rate of change in respiratory effort during an event in a given stage based on the time of day. This postulation is supported by the findings of Sforza et al. (Sforza *et al.*, 1998) but is in contrast to the findings of Kimoff and colleagues who reported that the rate of change of respiratory effort modified by pressure changes that activate upper airway mechanoreceptors, or increases in central respiratory drive, was not altered by the time of day (Cala *et al.*, 1996;Montserrat *et al.*, 1996b). Likewise, variations in chemoreflex properties, coupled to a more collapsible airway and consequently an increased effective recruitment threshold, could result in an upsurge in respiratory effort immediately at the termination of an event and following re-establishment of airway patency (El-Chami *et al.*, 2015). The increase in effort following re-establishment of airway patency could lead to hypocapnia and the initiation of subsequent events upon re-establishing the sleep state

(Dempsey *et al.*, 2010;Mateika & Narwani, 2009;Mateika & Syed, 2013b). This postulation is supported indirectly by Kimoff and colleagues (Cala *et al.*, 1996;Montserrat *et al.*, 1996b) who showed that the respiratory effort immediately prior to termination of the event was greater in the morning compared to the evening. To test the hypotheses stated above respiratory effort during events, immediately prior to termination of events, and closely after establishing airway patency was measured during N1 and N2 of non-rapid eye movement sleep in the evening and morning in individuals with obstructive sleep apnea.

Methods

Protocol

Findings discussed in this chapter resulted from the same visits we conducted to measure the frequency and duration of breathing events at different times of the day. For a detailed description of the protocol and instrumentation, refer to the protocol section of Chapter 2.

Data analysis

All polysomnography studies were analyzed for sleep stage, arousals, and respiratory-related events in accordance with standard published criteria. An apnea was identified as an event in which there was a cessation of inspiratory airflow for a minimum of 10 s. An event accompanied by a > 50% reduction in airflow that lasted for a minimum of 10 s and was accompanied by either an arousal, or a ≥ 3% reduction in oxygen saturation in the absence of an arousal, was classified as a hypopnea. The duration and number of apneas and hypopneas, along with the decrease in oxygen saturation that occurred during each event, was established for N1 and N2 of non-rapid eye movement sleep for each sleep session completed during Visit 4. The rate of change of upper airway inspiratory pressure from the start to the end of each breathing event (Δ pressure ÷ Δ time) detected during N1 and N2 sleep was determined (Figure 14). Analyses of events recorded in N3 were excluded from the results because the total amount of

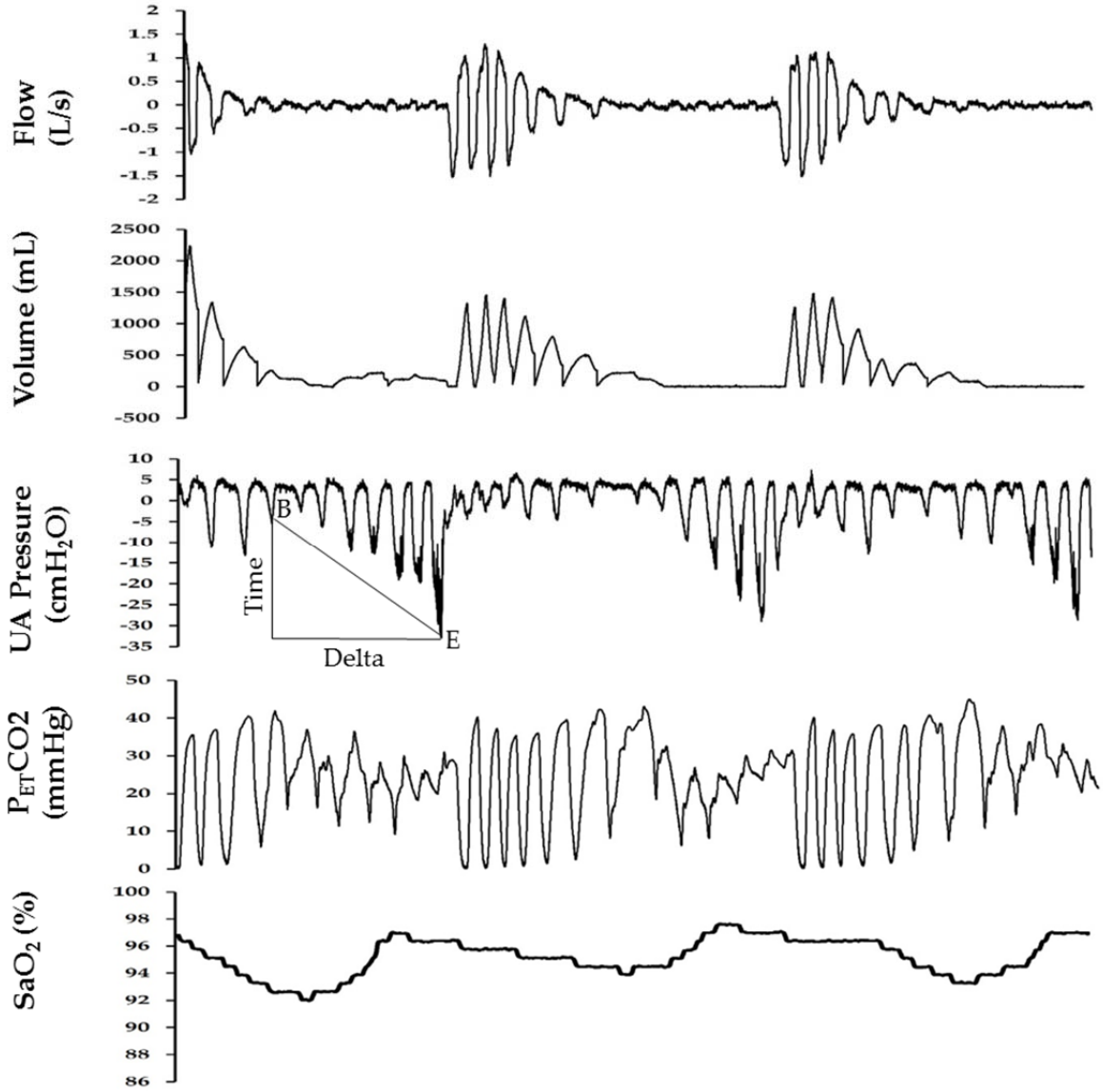


Figure 14. A raw figure showing the various parameters analyzed during an apneic event appearing in non-rapid eye movement sleep. Note that upper airway pressure was measured at the beginning and immediately prior to the termination of an event (see upper airway pressure – B and E). In addition, the difference between these measures (see upper airway pressure – delta) divided by the duration of the event was used to determine the rate of change of the respiratory effort during an event. Lastly, note that immediately following the termination of an event the maximum tidal volume and the lowest partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) within the initial 3 breaths after event termination was recorded.

time spent in N3 was insufficient to make comparisons between participants or sleep sessions. The lowest airway inspiratory pressure associated with the last respiratory attempt prior to termination of each event was also ascertained (Figure 14). Lastly, the tidal volume response of

the greatest magnitude, coupled with the accompanying fractional concentration of end-tidal carbon dioxide, was recorded from 1 of the initial 3 breaths after termination of a breathing event (Figure 14).

The decrease in carbon dioxide associated with the breath of the greatest magnitude following a breathing event was subtracted from baseline measurements of the partial pressure of end-tidal carbon dioxide measured from a 2-min period of stable non-rapid-eye-movement sleep during the evening and morning sleep sessions completed during visit 5 or 6 (El-Chami *et al.*, 2015;El-Chami *et al.*, 2014b). Baseline measures were obtained from these visits because a patent airway was established with continuous positive airway pressure in contrast to visit 4 in which a stable baseline period was not available to obtain baseline measures of the fractional concentration of carbon dioxide.

Statistical analysis

A two-way repeated measures ANOVA in conjunction with Fisher's least square difference post hoc test was used to compare the rate of change of respiratory effort, maximum effort immediately prior to event termination as well as the tidal volume and partial pressure of end-tidal carbon dioxide immediately after establishing airway patency. The two factors in the analysis were sleep stage (i.e. N1 and N2) and time of day (i.e. evening and morning). A paired t-test was used to compare the difference in the partial pressure of end-tidal carbon dioxide induced by hyperventilation and carbon dioxide measures that demarcated the apneic threshold obtained during N2 in the evening and morning. The end-tidal carbon dioxide measures were standardized to baseline measures.

Results

Table 4 shows the anthropometric variables obtained for the group. 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 4). The nadir of core body temperature was evident in the early morning during visit 4. Participants spent 73.4 ± 4.9 % and 78.0 ± 2.7 % of the total session time (i.e. 3 hours) in non-rapid eye movement sleep in the evening and morning ($P \geq 0.47$) during visit 4. The percentage of time spent in N1 or N2 for a given 3-hour sleep session was not significantly different between the evening and morning ($P \geq 0.85$).

Table 4: Baseline anthropometric, blood pressure and sleep measures

Variable	
Age, yr.	29.6 ± 2.3
Height, cm	177.1 ± 2.1
Weight, kg	83.8 ± 2.6
Body mass index, kg/m^2	26.6 ± 0.7
Systolic pressure, mmHg	118.7 ± 3.1
Diastolic pressure, mmHg	70.9 ± 3.0
Epworth Sleepiness Scale	10.0 ± 1.2
Apnea/hypopnea index, events/hr	44.4 ± 5.2
Lowest oxygen desaturation during apnea, %	86.5 ± 1.3
Race	6 AA, 3 Caucasian, 1 Asian, 1 Indian

Values are means ± SE

Respiratory effort was measured from an average of 48.8 ± 10.2 events during N1 and from 178.1 ± 25.2 events during N2 in each participant. The rate of change of respiratory effort during N1 compared to N2 was not significantly different ($P = 0.83$) (Figure 15A). Conversely, the upper airway inspiratory pressure associated with the last respiratory attempt prior to event termination was greater during N2 compared to N1 ($P \leq 0.04$) (Figure 15B). This increase was accompanied by a greater decrease in oxygen saturation in N2 compared to N1 ($P \leq 0.002$) (Figure 15C). Likewise, following event termination tidal volume was greater ($P \leq 0.03$) (Figure

15D) and the partial pressure of end-tidal carbon dioxide was less ($P \leq 0.05$) (Figure 15E) in N2

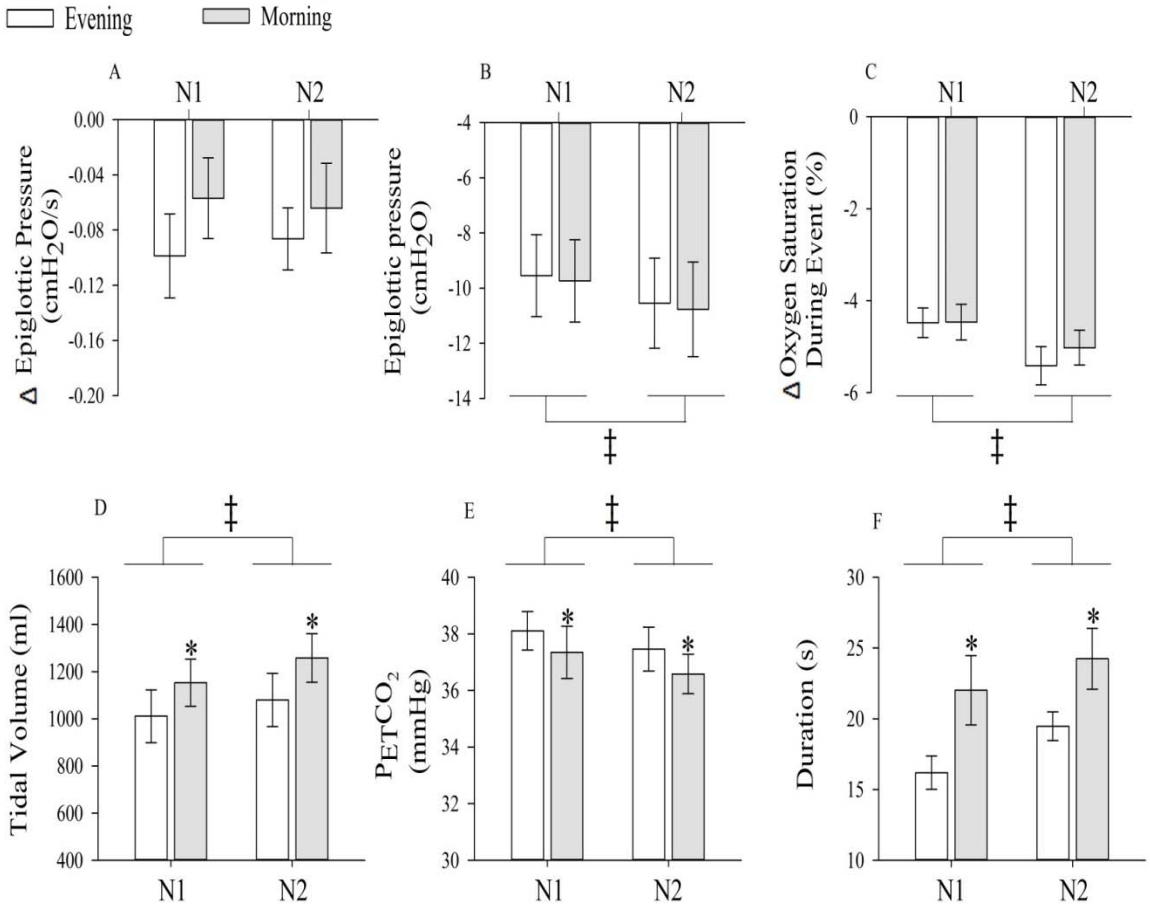


Figure 15. Histograms (mean ± S.E.) showing the rate of change in epiglottic pressure (A), epiglottic pressure at the termination of an event (B), change in oxygen saturation during events (C), maximum tidal volume immediately following an event (D), lowest partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) immediately following an event (E) and duration of breathing events (F) during N1 (white histograms) and N2 (gray histograms) of non-rapid eye movement sleep. ‡ significantly different from N1; * significantly different from the evening.

compared to N1. Respiratory effort during events and the magnitude of tidal volume after event termination was unrelated to the degree of flow limitation during hypopneic events which was similar in N1 compared to N2 (48.1 ± 2.2 vs. 47.4 ± 2.4 % of baseline, P = 0.34). Likewise, the number of events associated with an arousal at event termination (expressed as a percentage of the total number of events) was similar in N1 compared to N2 (77.3 ± 4.4 vs. 82.9 ± 4.0 % of events, P = 0.18). Thus, the presence or absence of an arousal at the termination of an event

likely did not account for differences in post event measures of tidal volume. The increase in respiratory effort during and following events in N2 were coupled to an increase in event duration during N2 compared to N1 ($P \leq 0.004$) (Figure 15F).

The rate of change of respiratory effort was similar in the evening compared to the morning ($P = 0.34$) (Figure 15A), as was the upper airway inspiratory pressure measured for the last respiratory event prior to event termination ($P = 0.88$) (Figure 15B) and the decrease in oxygen saturation ($P = 0.13$) (Figure 15C). In contrast, following event termination, tidal volume was greater ($P \leq 0.01$) (Figure 15D) and consequently the partial pressure of end-tidal carbon dioxide was less ($P \leq 0.05$) (Figure 15E) in the morning compared to the evening independent of sleep stage. The decrease in the partial pressure of carbon dioxide in response to hyperventilation in the morning was below the apneic threshold compared to the partial pressure of end-tidal carbon dioxide which remained slightly above the apneic threshold in the evening ($P \leq 0.02$) (Figure 16). The duration of events was greater in the morning compared to the evening independent of sleep stage ($P \leq 0.001$) (Figure 15F).

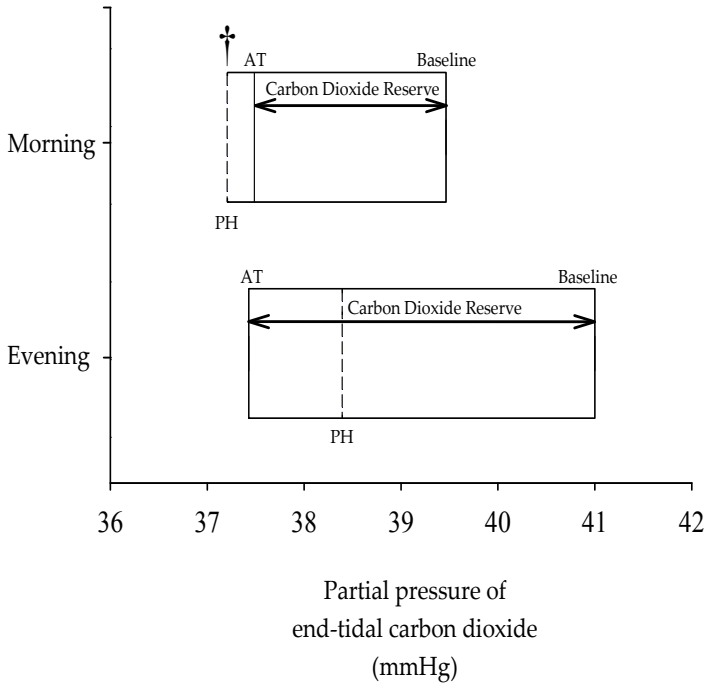


Figure 16. Histograms which show the group mean values calculated from baseline measures of the partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$), the $P_{ET}CO_2$ that demarcates the apneic threshold (AT) which was previously published and the lowest $P_{ET}CO_2$ measured immediately following breathing events in the present investigation (dashed line; PH–post hyperventilation). Note that in the morning the $P_{ET}CO_2$ initiated by post hyperventilation was below the apneic threshold in contrast to the evening. † $P_{ET}CO_2$ initiated by PH standardized to the AT significantly different in the morning compared to the evening.

Discussion

We discovered that the rate of change of respiratory effort during breathing events was similar throughout N2 and N1, even though the duration of events was greater in N2 compared to N1. Coupled to the increase in duration the respiratory effort immediately prior to and after termination of an event was greater in N2 compared to N1. The increase in respiratory effort (i.e. tidal volume) after termination of an event was coupled to a reduced partial pressure of end-tidal carbon dioxide. We also found that within a given stage of non-rapid eye movement sleep (i.e. N1 or N2) the rate of change of respiratory effort and the maximum respiratory effort immediately prior to termination of an event was similar in the morning compared to the evening, whereas the duration of breathing events was greater in the morning. Despite the similarity in respiratory effort during events, tidal volume was greater and the partial pressure of end-tidal carbon dioxide was reduced once airway patency was re-established in the morning compared to the evening.

Mechanisms responsible for the increase in event frequency & duration in N2 compared to N1

In our previous investigation we found that the duration of breathing events was increased during N2 compared to N1 of non-rapid eye movement sleep. At least two mechanisms could be responsible for the increase as outlined above (see *Introduction*). The increase could be caused by a rise in the arousal threshold along with an increase in the effective recruitment threshold for upper airway muscle activity. Alternatively, a blunted respiratory response to afferent inputs (e.g. chemoreflex and mechanoreceptor inputs) reflected by a decrease in the rate of change of respiratory effort during apneic events in N2 could be responsible for sleep stage related difference in apnea duration. Kimoff and colleagues reported that the rate of change of respiratory effort during breathing events in N2 is independent of event duration (Cala *et al.*, 1996;Montserrat *et al.*, 1996b). If this finding is consistent across sleep

states then a similar rate of change of respiratory effort during events in N2 compared to N1 would be expected. This expectation was confirmed by our results which showed that the rate of change of respiratory effort was similar in N2 compared to N1. In contrast, we showed that the respiratory effort immediately prior to termination of events in N2 was greater compared to N1. Thus, our results imply that increases in the arousal threshold and effective recruitment threshold had a role in the prolongation of event duration during N2 compared to N1.

Our results also showed that the magnitude of the tidal volume response was greater and the partial pressure of end-tidal carbon dioxide was reduced immediately following termination of breathing events in N2 compared to N1. The increase in the tidal volume response was independent of the degree of flow limitation and the number of events accompanied by arousal. Thus, we propose that an elevated arousal threshold leading to an increase in event duration during N2 was accompanied by enhancement of respiratory stimuli that contributed to the augmented tidal volume response evident following termination of events in N2. This suggestion is supported by our results which showed a greater decrease in oxygen saturation during events in N2. Consequently, termination of an event was likely accompanied by increased chemoreceptor input (Pillar *et al.*, 2000;Loewen *et al.*, 2011b;Nicholas *et al.*, 2010;Saboisky *et al.*, 2010b;Stanchina *et al.*, 2002), along with other sensory inputs (Berry *et al.*, 1995;Cala *et al.*, 1996;McNicholas *et al.*, 1987), that initiated increases in tidal volume coupled with reductions in the partial pressure of end-tidal carbon dioxide that exceeded measures following events in N1. Independent of the mechanisms responsible for the increased end-apnea ventilatory response the accompanying hypocapnia could increase the likelihood of perpetuating apneic events in N2 compared to N1 (Dempsey *et al.*, 2010;Mateika & Narwani, 2009;Mateika & Syed, 2013b). This suggestion is supported by our previous findings which showed that the frequency of breathing events was increased in the evening and afternoon in N2 compared to N1

of non-rapid eye movement sleep (El-Chami *et al.*, 2015). The presence of hypocapnia at the onset of subsequent events may have also contributed to prolonging event duration; since additional time would be required to reach a level of carbon dioxide that was sufficient to terminate events in N2 compared to N1.

Mechanisms responsible for the increase in event frequency and duration in the morning compared to the evening

We previously reported that the frequency of events increased in the morning compared to the evening and afternoon in N1 (El-Chami *et al.*, 2015). This difference was also evident in a subgroup of participants (n = 5) during N2 (El-Chami *et al.*, 2015). As shown in the present investigation (see Figure 15) and in our previously published work (El-Chami *et al.*, 2015) the duration of breathing events increased in the morning compared to the evening independent of the stage of non-rapid eye movement sleep.

The increase in event duration in the morning compared to the evening in both N1 and N2 suggest that the arousal threshold along with the effective recruitment threshold of the upper airway muscles following airway collapse was higher in the morning compared to the evening. This suggestion is supported by previous results which showed that the respiratory effort immediately prior to termination of an event in N2 was increased in the morning compared to the evening, while the rate of change in respiratory effort during breathing events was not altered by the time of day (Cala *et al.*, 1996;Montserrat *et al.*, 1996b). We also found that the rate of change of respiratory effort was similar during events within N1 and N2 in the evening and morning. However, in contrast to previously published findings (Cala *et al.*, 1996;Montserrat *et al.*, 1996b) we found that the maximum respiratory effort at the termination of events was similar in the evening and morning within N1 and N2. Thus, alterations in the arousal threshold or the sensitivity of the respiratory response to afferent inputs during events may not provide the sole

explanation for increases in event duration in the morning compared to the evening. We propose that modulation of the carbon dioxide reserve, coupled to differences in metabolic rate linked to the time of day contribute to the prolongation of event duration in the morning.

Earlier we showed that an increase in chemoreflex sensitivity coupled to a reduction in the carbon dioxide reserve was evident in the morning compared to the evening (El-Chami *et al.*, 2014b). The increase in chemoreflex sensitivity likely contributed to the increase in tidal volume and the decrease in the partial pressure of end-tidal carbon dioxide that was evident in the morning compared to the evening within N1 or N2 in the present investigation. Moreover, the increase in chemoreflex sensitivity coupled to a decrease in the carbon dioxide reserve in the morning likely ensured that the level of hypocapnia relative to the apneic threshold was greater at the onset of an event in the morning compared to evening. Indeed, measures of end-tidal carbon dioxide immediately following event termination in the present investigation, coupled to previously published measures of the apneic threshold (El-Chami *et al.*, 2014b), showed that the partial pressure of carbon dioxide in response to hyperventilation in the morning dropped below the apneic threshold but remained above it in the evening. Given that studies have shown that the response to hypoxia is blunted or absent in the presence of hypocapnia (Rapanos & Duffin, 1997b;Weil *et al.*, 1970b;Wilson & Day, 2013b), it is possible that hypoxia does not significantly enhance receptor feedback prior to the abolition of hypocapnia, despite enhanced chemoreflex sensitivity. In addition, activation of chemoreflex inputs was likely reduced by a decrease in metabolic rate in the morning. We have shown that core body temperature, minute ventilation and end tidal carbon dioxide are reduced in the morning compared to the evening (El-Chami *et al.*, 2014b). In addition, in the present study decreases in oxygen saturation were similar in the morning compared to the evening even though event duration was longer in the morning compared to the evening. Thus, the presence of hypocapnia at the onset of events coupled with

the reduced metabolic rate in the morning could delay the stimuli (i.e. hypoxia and hypercapnia) required to activate the arousal and effective recruitment threshold, resulting in an increased event duration. Likewise, the increased probability of carbon dioxide levels being less than values that demarcate the apneic threshold would likely contribute to increasing the frequency of apneic events (Dempsey *et al.*, 2010;Mateika & Narwani, 2009;Mateika & Syed, 2013b).

Conclusion

We conclude that alterations in the arousal threshold, reflected by an increase in respiratory effort at event termination, coupled to increases in tidal volume and reductions in $P_{ET}CO_2$ contribute to modifications in event duration and frequency associated with variations in sleep state or time of night.

CHAPTER 5 - MILD INTERMITTENT HYPOXIA WITH SUSTAINED HYPERCAPNIA REDUCES THERAPEUTIC CPAP AND IMPROVES AIRFLOW IN PARTICIPANTS WITH OBSTRUCTIVE SLEEP APNEA

Introduction

Long-term facilitation is characterized by a sustained increase in respiratory motor activity following exposure to mild intermittent hypoxia (Mateika and Syed, 2013). A sustained increase in motor activity has been recorded at sites within the medulla and spinal cord that control ventilation and at sites in the medulla responsible for the control of upper airway muscles (Mateika and Fregosi, 1997; Babcock and Badr, 1998; Trumbower *et al.* 2012). Long-term facilitation of phrenic motor nerve activity and ventilation has been initiated in a variety of species including healthy humans, humans with sleep apnea and spinal cord injured humans (Aboubakr *et al*., 2001; Fuller *et al.,* 2003, Wadhwa *et al.*, 2008; Devinney *et al.* ,2013; Tester *et al*. 2014). Likewise, long-term facilitation of the hypoglossal nerve or the genioglossus muscle has been observed in a number of animals including humans (Fuller *et al., 2001*; Fuller, 2005; Chowdhuri *et al.,* 2008; McKay *et al.,* 2004). Given that long-term facilitation of upper airway muscle activity can be initiated by intermittent hypoxia we and others hypothesized that the initiation of this phenomenon might improve upper airway patency in humans with sleep apnea (Mateika and Fregosi, 1997; Babcock and Badr, 1998; Aboubakr *et al*., 2001). This led to the possibility that apnea severity across the night might improve as a consequence of natural exposure to intermittent hypoxia. However, a number of studies have revealed that apnea severity increases rather than decreases from evening to morning (Fanfulla *et al.* 1997; Cala *et al,* .1996; Sforza *et al.,* 1998). We have addressed the potential reasons for the lack of influence of long-term facilitation on apnea severity in previous reviews (Mateika and Syed, 2013 ; Mateika *et al.*, 2015 ; Mateika and Komnenov, 2016). Briefly there are a variety of mechanisms (i.e. chemoreflex sensitivity, carbon dioxide reserve, airway inflammation) that are modulated

across the night that likely prevent or predominate over the influence potentially exerted by long-term facilitation of upper airway muscle activity on airway collapsibility. Consequently, we postulated that if these detrimental influences were minimized or eliminated then the effects of long-term facilitation might manifest more clearly. More specifically we reasoned that improved upper airway patency via the initiation of long-term facilitation could potentially serve to reduce the positive airway pressure required to treat individuals with sleep apnea. This reduced pressure could improve treatment compliance, which is reportedly limited (Sawyer *et al*., 2011), and ultimately the co-morbidities (i.e. cardiovascular, metabolic and neurocognitive) linked to sleep apnea. This possibility coupled to the direct effects of intermittent hypoxia on these co-morbidities that have been reported could serve as novel adjunct therapy to treat sleep apnea and its multiple co-morbidities. Thus, the present investigation was designed primarily to determine if long-term facilitation of upper airway muscle activity initiated by exposure to intermittent hypoxia serves to reduce the therapeutic positive airway pressure required to maintain airway patency in individuals with obstructive sleep apnea.

Long-term facilitation is mediated by at least two cellular pathways deemed the Q and S pathway (DeVinney *et al*., 2013). It is has been hypothesized that the Q pathway is initiated by mild intermittent hypoxia. This pathway is regulated in part by serotonin in addition to other neuromodulators. Serotonin along with the proteins and kinases that have been identified within the Q pathway are reportedly modulated by a circadian rhythm (Agren et al., 1986, Mateos et al., 2009, Sun et al., 2002, Serchov and Heumann, 2006). If so, then the magnitude of long-term facilitation would be expected to vary according to the time of day. Indeed we previously reported that the magnitude of long-term facilitation of ventilation in humans was greater in the evening compared to the morning (Gerst *et al.*, 2011). Thus, a secondary aim of this investigation was designed to determine if the impact of long-term facilitation on the therapeutic

positive pressure required to treat obstructive sleep apnea was modulated by the time of day.

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 completed the protocol by visiting the laboratory on six different occasions (Figure 17). On the first visit, informed consent was obtained from participants before completion of a physical examination and health and lifestyle questionnaires. In addition, blood pressure, lung volumes and a 12-lead ECG were obtained to confirm the absence of comorbidities (i.e. heart and lung disease, hypertension, and obesity). During the second visit, participants completed a diagnostic nocturnal polysomnogram to determine the presence of obstructive sleep apnea. Once confirmed participants maintained a regular sleep cycle at home for a two week period to ensure a consistent circadian rhythm. Sleep/wake times were monitored via an actigraph watch (Actiwatch Spectrum, Philips Respironics, Murraysville, PA). During this two week period, participants returned to the laboratory for an overnight visit (i.e. visit 3) to acclimate to continuous positive airway pressure ventilation and to determine the therapeutic pressure required to eliminate breathing events.

Figure 17. A schematic diagram showing the protocol completed by the participants. * Sham protocol was conducted in the evening and morning only. † Administration of IH was randomized to evening or morning

After the two week period, participants returned for a 4th visit to complete a 21-hour constant routine protocol (Figure 17). Participants arrived at the laboratory at 8 pm and completed 3 separate sleep sessions from 10 pm to 1 am, 6 am to 9 am and 2 pm to 5 pm. During each session the therapeutic continuous positive pressure airway pressure was established. Thereafter a sham protocol, emulating the timeline of the intermittent hypoxia intervention applied in visits 5 and 6 (see below for further for details), was completed. During this time interval the partial pressure of end-tidal oxygen and carbon dioxide remained unaltered. Following completion of the protocol the continuous positive airway pressure was reduced in 1-2 cmH_2O decrements to compare measures of respiratory parameters (see Data Analysis for details of respiratory parameters) at similar pressures following exposure to intermittent hypoxia and sustained hypercapnia.

During the 5 hours between sessions, participants were awake and in a semi-recumbent position. The room was dimly lit with a luminance of 30 lux, while the temperature and humidity were maintained between 22-24° C and 25-35%, respectively. The participants watched a movie (i.e. non-stimulating light comedies or documentaries) for two hours, followed by 90 minutes of reading, and 30 to 45 minutes of inactivity prior to the start of the setup for the following sleep session. The subjects received small snacks with a constant caloric content every 95 minutes (composition: 75 % carbohydrates, 15% protein and 10% fat) and a maximum of 1L of water. During completion of the protocol participants were isolated from external cues such as sunlight, TV, clocks, phones, computers, radios, and internet.

During visits 5 and 6 the therapeutic pressure for a given sleep session was established and baseline (i.e. B_1) measures of respiratory parameters (see Data Analysis for respiratory parameters measured) were obtained over a minimum of 10 minutes (Figure 18). The partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) was then increased by 3 mmHg and measures of

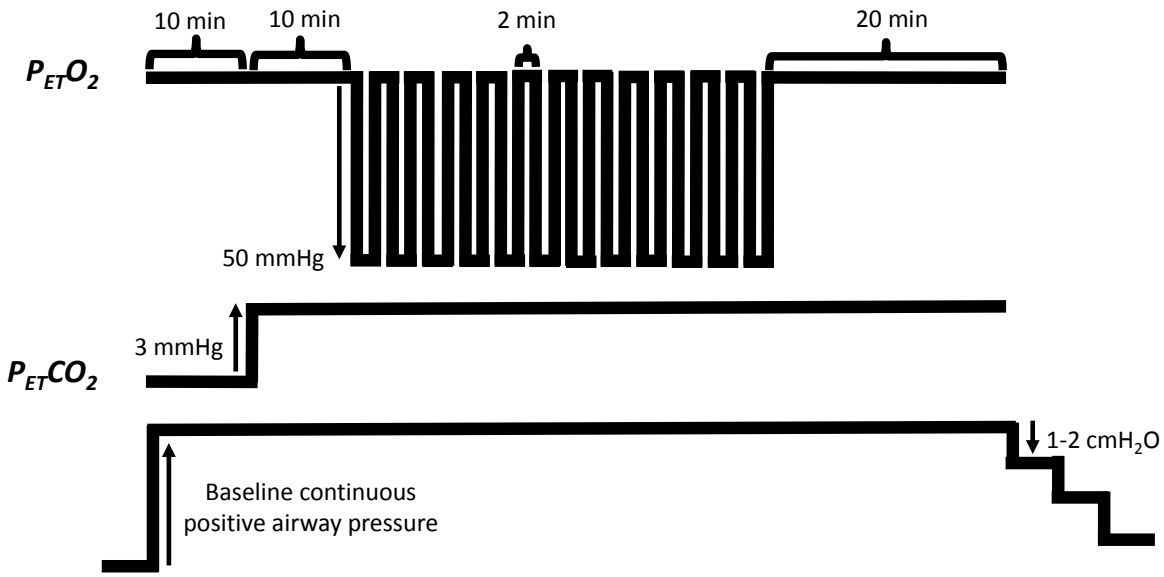


Figure 18. Diagram showing the severity and pattern of intermittent hypoxia. The tracings illustrates the changes in the partial pressure of end-tidal oxygen ($P_{ET}O_2$) (upper) and carbon dioxide ($P_{ET}CO_2$) (middle) and the alterations in continuous positive airway pressure throughout the protocol (bottom)

the respiratory parameters were obtained for an additional 10 minutes during the second baseline (B_2) period (Figure 18). Thereafter, participants inspired a gas mixture comprised of 8 % oxygen and balance nitrogen to achieve a partial pressure of end-tidal oxygen ($P_{ET}O_2$) (~ 55 mmHg) that resulted in an oxygen saturation of 88 % (Figure 20). Maintenance of the $P_{ET}O_2$ was achieved by the addition of 100 % oxygen into the circuit using a flowmeter. Participants were exposed to this gas mixture 12 times for two-minutes each time. The 12 hypoxic episodes were separated by 2 minute recovery intervals with the exception of the recovery period that followed the 12th hypoxic episode, which was 15 minutes in duration. For presentation purposes the 15 minute recovery period was divided into three 5 minute segments. Throughout the protocol, including the end recovery period, the $P_{ET}CO_2$ was maintained at 3 mmHg above baseline (Figure 18). After the 15 minute end-recovery period the continuous positive airway pressure was reduced in 1-2 cmH_2O steps. The participants were exposed to the IH and sustained hypercapnia during an evening (10 pm to 1 am) and morning session (6 am to 9 am) on separate occasions (Visits 5 and

6), which were randomized. When the participants were exposed to IH and sustained hypercapnia in the morning they were treated with continuous positive airway during the evening session without exposure to the hypoxic stimulus.

Instrumentation

During the 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 (Respitrace, 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 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 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

Nocturnal polysomnography. All polysomnography studies were analyzed in 30s epochs for sleep stage and arousals. In addition, during the second visit respiratory-related events were

scored according to standard published criteria. Apneas were defined as the cessation of inspiratory flow for 10 seconds or more, while hypopneas were characterized by a decrease in inspiratory flow of more than 50% accompanied by a 3% (or greater) blood oxygen desaturation and/or an arousal from sleep. The apnea hypopnea index (i.e. the average number of respiratory events per hour) was determined to ensure that the severity of sleep apnea fell within the inclusion criteria.

Intermittent hypoxia protocol. The physiological parameters measured throughout the protocol were ventilation, $P_{ET}CO_2$, $P_{ET}O_2$, SaO_2, heart rate, tidal volume and breathing frequency. Average absolute values of the parameters were obtained from the last 2 minutes of B_1, B_2, the last minute of each hypoxic and recovery period and the last two minutes of each decrement in continuous positive airway pressure. Select data were standardized to B_2 to compare results between the intermittent hypoxia and sham protocol. This analysis was completed because measures of minute ventilation and tidal volume were greater during B_2 of the intermittent hypoxia compared to the sham protocol because $P_{ET}CO_2$ was increased 3 mmHg above baseline during B_2 of the intermittent hypoxia protocol. Average values of tidal volume, airflow and upper airway resistance were determined from 10 breaths measured during the last minute of B_2. Data from the last minute of end recovery and from two step downs were standardized to B_2 measures. The selected step downs in pressure were equivalent to values of positive pressure that resulted in a 25 % and 50 % reduction in airflow during the sham protocol. Resistance was defined as the inverse of the average slope of the linear portion in the pressure/flow loop (Figure 19). In order to obtain the slope, commercial software (Matlab) was used to calculate the slope of a series of 3 sequential data points measured during inspiration prior to a plateau in airflow (Figure 19). The slopes were then averaged for each breath. Thereafter a 10 breath average was calculated for each selected period outlined above.

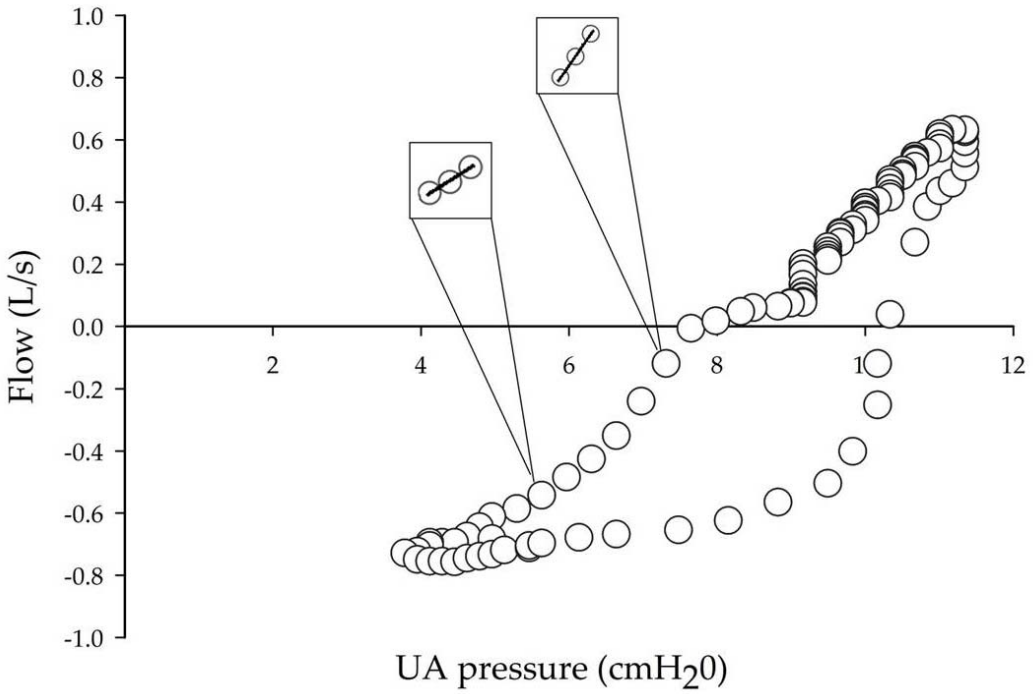


Figure 19. Figure showing the method used to measure upper airway resistance. A tangent line was fit to 3 consecutive points throughout the inspiratory portion of the breath prior to maximal flow to determine resistance (1/slope of the tangent line).

Sham protocol. The sham protocol was completed to compare the changes (or lack thereof) in the physiological parameters following exposure to the continuous positive airway pressure for a duration comparable to the one required to complete the intermittent hypoxia protocol. The comparison serves to ensure that the hypothesized improvement in upper airway and breathing stability were due to intermittent hypoxia and sustained hypercapnia. Analysis of the data measured during the sham protocols was identical to the analysis completed for the intermittent hypoxia trials (see explanation above).

Statistical analysis

A two way analysis of variance with repeated measures in conjunction with Student Newman Keuls post hoc test was used to compare data measured during B_2 of the intermittent and sham protocol. The factors in the design were "protocol" (intermittent hypoxia vs. sham) and "time of day" (evening vs. morning). A similar analysis was used to compare the data

within a given protocol (i.e. intermittent hypoxia or sham). The factors in the design were "time point" (B_2 vs. End Recovery 1, 2 & 3 vs. Step Down 1, 2, 3 & 4) and "time of day" (evening vs. morning). Lastly, this analysis was used to compare the standardized data across the intermittent hypoxia and sham protocols. The factors in the design were "time point" (B_2 vs. End Recovery vs. Step Down 25 % & 50 %) and "time of day" (evening vs. morning). Data are presented as means ± SE. $P \leq 0.05$ was considered statistically significant. $0.05 < P \leq 0.07$ was considered to be characteristic of a trend toward significance.

Results

Table 5 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. The average therapeutic pressure required to eliminate apnea during sleep on *visit 3* in the obstructive sleep apnea participants was 11 ± 0.7 cmH_2O.

Table 5. Baseline anthropometric, blood pressure and sleep measures

Variable	
Age, yr.	25.2 ± 2.2
Height, cm	178.3 ± 5.5
Weight, kg	83.6 ± 3.1
Body mass index, kg/m^2	25.7 ± 0.9
Systolic pressure, mmHg	120.1 ± 3.2
Diastolic pressure, mmHg	72.2 ± 3.3
Epworth Sleepiness Scale	8.3 ± 1.4
Apnea/hypopnea index, events/hr	37.03 ± 5.2
Lowest oxygen desaturation during apnea, %	88.8 ± 1.4
Race	7 Caucasian ,2 AA , 1 Indian

Values are means ± SE

The therapeutic pressure required to eliminate apnea during B_2 of the sham protocol in the evening and the morning was 11.2 ± 0.6 and 12.4 ± 0.5 cmH_2O, respectively. The therapeutic pressure required to eliminate apnea during B_2 of the intermittent hypoxia protocol in the evening and the morning was 12.1 ± 0.7 and 13.1 ± 0.7 cmH_2O, respectively. The therapeutic pressure required to eliminate apnea was significantly less in the evening compared to the morning during the intermittent hypoxia and sham protocol ($P < 0.02$). The therapeutic pressure during B_2 of the intermittent hypoxia protocol tended to be increased compared to the therapeutic pressure measured at the same time point during the sham protocols in the evening and morning ($P < 0.06$).

Measures of the physiological parameters obtained during B_1 and B_2 of the sham and intermittent hypoxia protocols are shown in Figure 20. Note that by design $P_{ET}CO_2$ during B_2 was greater during the intermittent hypoxia compared to the sham protocols ($P < 0.005$). Consequently, minute ventilation ($P < 0.001$) and tidal volume ($P < 0.001$) were greater during B_2 of the intermittent hypoxia compared to the sham protocols.

Following exposure to intermittent hypoxia minute ventilation ($P < 0.001$) and tidal volume ($P < 0.001$) during end-recovery and the step-downs in positive pressure were greater compared to B_2 ($P < 0.001$) (Figure 20). Consequently, $P_{ET}O_2$ was greater during the end-recovery period following exposure to intermittent hypoxia compared to B_2 ($P < 0.01$) (Figure 20). The increases observed following exposure to intermittent hypoxia were evident even though the therapeutic pressure ($P = 0.4$) and $P_{ET}CO_2$ ($P = 0.3$) during the end-recovery periods were similar to measures obtained during B_2. The increase in minute ventilation tended to be greater ($P = 0.07$) and the increase in tidal volume was greater ($P = 0.04$) in the evening compared to the morning (Figure 20). Following exposure to the sham protocol minute ventilation ($P > 0.7$) and tidal volume ($P > 0.3$) during end–recovery and the step-downs in

positive pressure were similar to B_2.

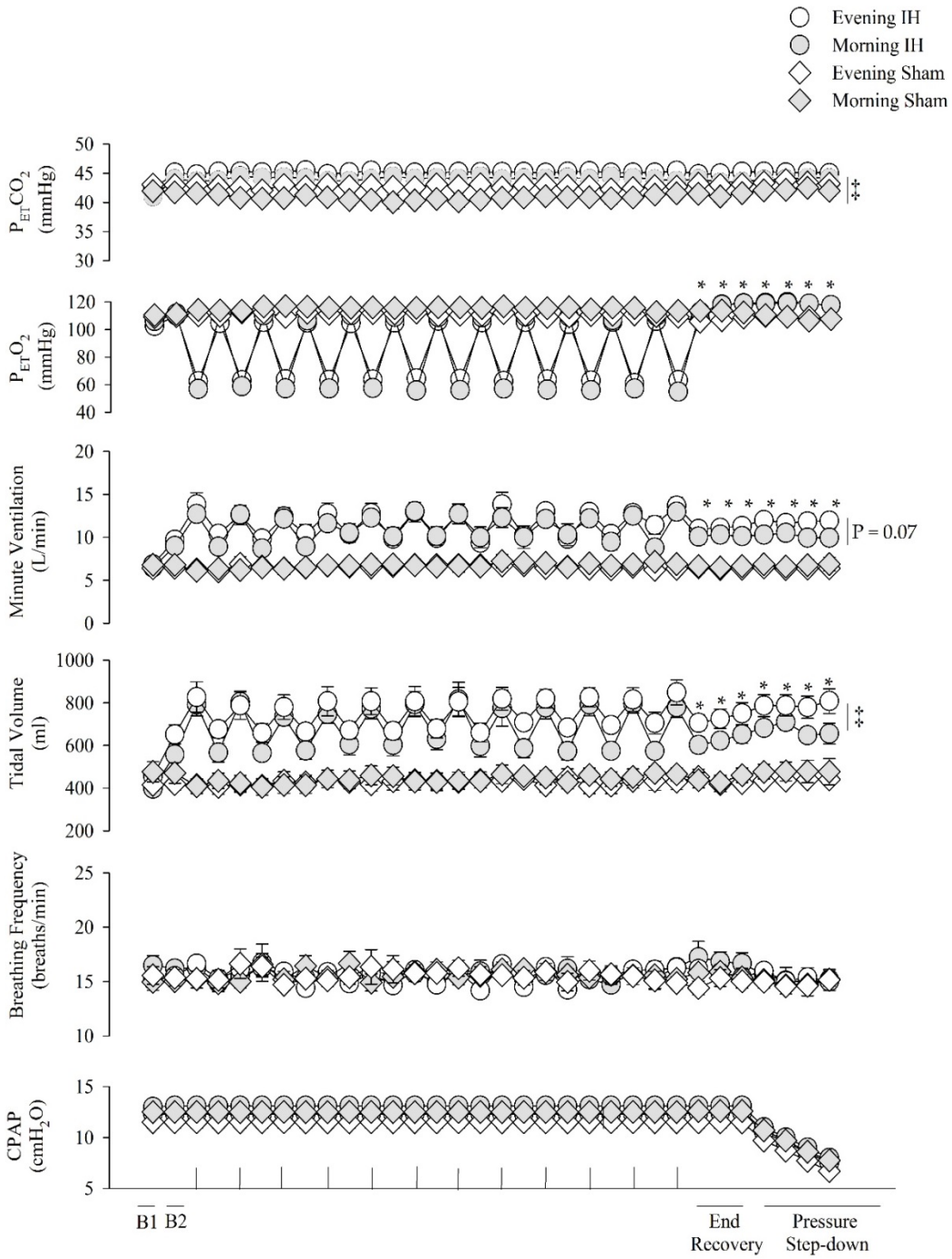
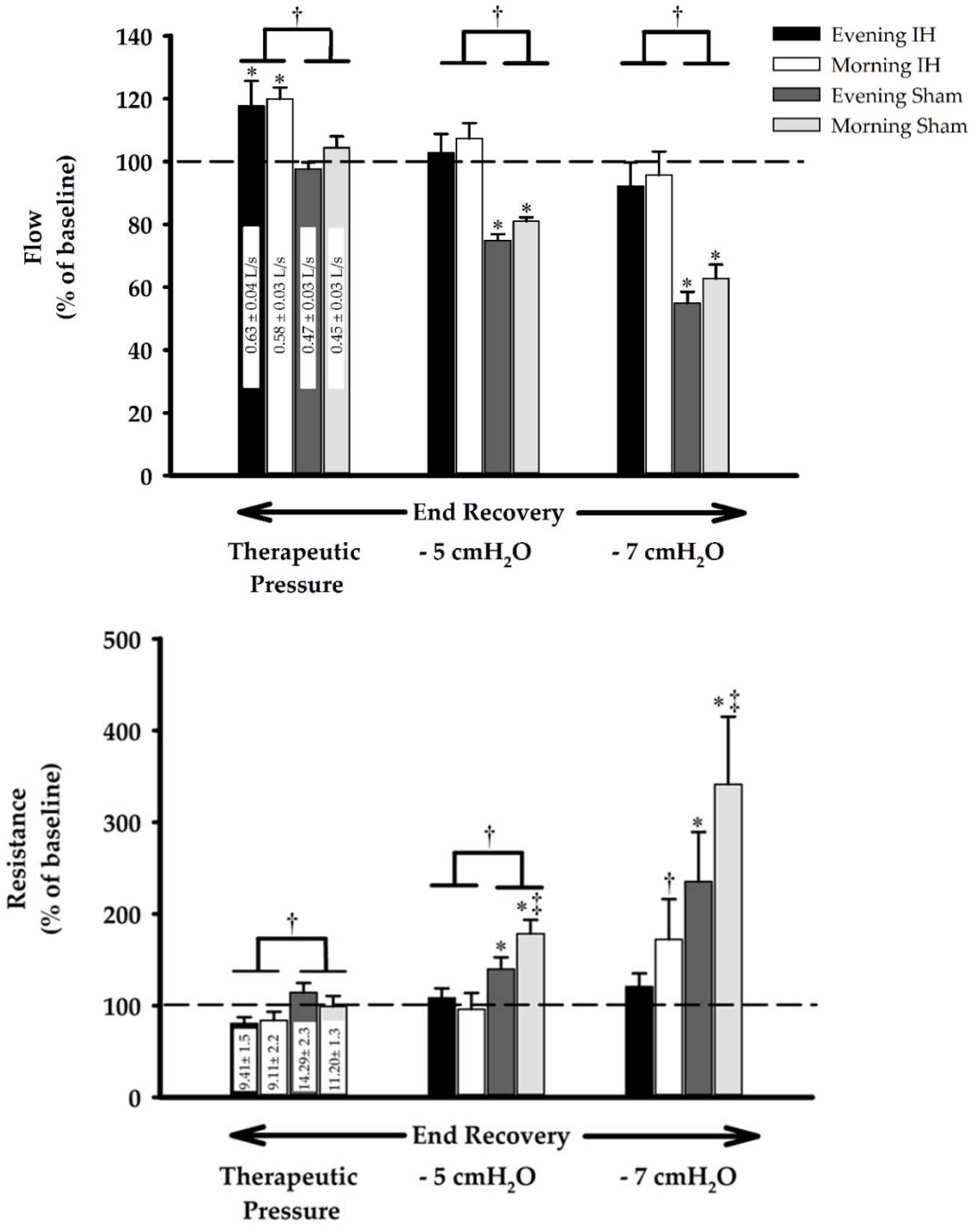


Figure 20. Scatter plots showing changes in end-tidal $P_{ET}CO_2$, $P_{ET}O_2$, minute ventilation, tidal volume, blood oxygen saturation (SaO_2), breathing frequency, and continuous positive airway pressure throughout the intermittent hypoxia and sham protocols. *significantly different than baseline ; ‡significantly different than the evening.

As expected the increase in tidal volume observed during the end recovery period was coupled to an increase in inspiratory flow following exposure to intermittent hypoxia ($P < 0.003$) but not after the sham protocol (Figure 21). Likewise, airway resistance during the end recovery period was significantly reduced following exposure to intermittent hypoxia compared to the sham protocols ($P < 0.03$). The increase in inspiratory flow and the decrease in resistance following exposure to intermittent hypoxia during the end recovery period were similar in the evening and the morning. Inspiratory flow and resistance remained at baseline levels following exposure to intermittent hypoxia after the therapeutic positive airway pressure was reduced by 5 cm H_2O (Figure 21). In contrast, after completion of the sham protocol inspiratory flow was reduced ($P < 0.001$) and resistance was increased ($P < 0.01$) in response to the 5 cm H_2O reduction in positive airway pressure (Figure 21). After the positive airway pressure was reduced by 7 cm H_2O following exposure to intermittent hypoxia, inspiratory flow was reduced and resistance was greater compared to baseline in some participants but not others. Consequently, these measures were not significantly different from baseline. In contrast, inspiratory flow was reduced and resistance were greater than baseline following the sham protocol ($P < 0.04$) (Figure 21). The changes in inspiratory flow after the 5 and 7 cm H_2O reduction in positive airway pressure during recovery from the intermittent hypoxia and sham protocols were similar in the evening and morning (Figure 21). In contrast, resistance measures were greater in the morning compared to the evening after the positive airway pressure was reduced by 5 and 7 cm H_2O following the sham protocol (see 5 and 7 cm H_2O) ($P < 0.02$) (Figure 21).



Baseline resistance values in cmH2O/L/s

Figure 21. Histograms showing airflow (top) and upper airway resistance (bottom) after completion of the intermittent hypoxia or sham protocols. Measures were obtained at baseline therapeutic pressure and following decreases in pressure that corresponded to a 25 and 50% decrease in airflow during the sham protocol. Note that despite the decrease in pressure, flow and resistance were not significantly altered compared to baseline and the sham protocols. *significantly different than baseline; †significantly different than sham; ‡significantly different than the evening.

Discussion

We confirmed our previous findings that exposure to mild intermittent hypoxia initiates long-term facilitation of minute ventilation during sleep in individuals treated with continuous positive airway pressure. Novel findings from our investigation indicated that exposure to mild intermittent hypoxia initiates long-term facilitation of upper airway muscle activity. The initiation of this phenomenon was reflected by an increase in airflow and a reduction in airway resistance during treatment with positive airway pressure following exposure to intermittent hypoxia. Moreover, airflow and airway resistance were maintained at baseline levels even after positive airway pressure was reduced below therapeutic values. In addition to our primary findings, our secondary findings revealed that upper airway patency could be modulated by a circadian rhythm, which supports our previous findings. More specifically the therapeutic pressure required to maintain airway patency was higher in the morning compared to the evening during completion of the sham protocols. Likewise, a reduction in positive airway pressure below therapeutic values (i.e. 7 cm H_2O) resulted in an increase in airway resistance which was greater in magnitude in the morning compared to the evening.

Critique of experimental protocol

In the present investigation mild intermittent hypoxia was used to initiate respiratory plasticity. The use of intermittent hypoxia as a therapeutic modality is counterintuitive to the findings from a number of animal studies which suggested that intermittent hypoxia causes autonomic, metabolic and neurocognitive dysfunction (Fletcher 1995, 1997, 2000 and 2001). However, to our knowledge there is little to no evidence that mild intermittent hypoxia leads to these detrimental outcomes which have been linked to long-term exposure to severe levels of hypoxia. Instead, a number of reviews, with the support of published research findings, have convincingly outlined that numerous beneficial outcomes might be linked to exposure to mild

intermittent hypoxia (Mateika *et al.*,2015; Mateika and Komnenov, 2016; Serebrovskaya *et al.*, 2008). However, we did report previously that some forms of respiratory plasticity (i.e. progressive augmentation) initiated by acute exposure to mild intermittent hypoxia promotes breathing instability particularly when other forms of plasticity that could be beneficial (i.e. long-term facilitation) are neutralized (Yokhana *et al.*, 2012). Therefore we proposed that the potential beneficial effects of long-term facilitation might only manifest if the detrimental forms of plasticity are eliminated or mitigated. Thus we proposed that the beneficial effects of long-term facilitation will be clearly evident once the influence of progressive augmentation was mitigated or eliminated with continuous positive airway pressure.

In our investigation intermittent hypoxia was combined with a level of carbon dioxide that was sustained slightly above baseline levels. The maintenance of carbon dioxide throughout the intermittent hypoxia protocol was employed to ensure that long-term facilitation of ventilation clearly manifested itself throughout the protocol; since we previously established that long-term facilitation typically does not manifest in the presence of hypocapnia (Mateika and Sandhu, 2011). Given the employment of carbon dioxide levels that were above baseline it could be argued that the long-term facilitation that was evident, and coupled to reduced upper airway resistance and collapsibility, was induced principally by forms of respiratory induced by increased levels of carbon dioxide. However, we previously completed control experiments which showed that the magnitude of long-term facilitation of ventilation was determined wholly or principally by exposure to intermittent hypoxia and not sustained hypercapnia (Harris *et al.*, 2006). Moreover, we showed that repeated daily exposure to elevated levels of carbon dioxide did not lead to long-term facilitation of minute ventilation. In contrast, repeated daily exposure to intermittent hypoxia led to a progressive increase in the magnitude of long-term facilitation.

It has been standard practice to measure airway resistance as the inverse of the slope at a

specific point on the linear portion of the flow-pressure curve. However, given the complex dynamic nature of the airway during inspiration the customary method neglects the progression of airway mechanics and thus measures only reflect the initial portion of inspiration. Therefore airway resistance at points closer to termination of inspiration is not reflected in the measurement. The method we used reflects the average resistance measured throughout the inspiratory phase prior to a plateau in inspiratory flow. It is reasonable to suggest that the method we employed does not control for the potential impact of a number of variables that might influence airway resistance (e.g. lung volume). Although this is a viable argument, measuring upper airway resistance throughout inspiration accurately reflects the impact of long-term facilitation on this variable.

Long-term facilitation of minute ventilation and its components

The results from the present study largely confirm our previous findings that exposure to mild intermittent hypoxia induces long-term facilitation of minute ventilation during sleep. Likewise, our results confirm that modulation of tidal volume was principally responsible for the observed long-term facilitation. We were also interested in examining if long-term facilitation of minute ventilation was modulated by the time of day. Previously we reported that the magnitude of long-term facilitation during wakefulness was greater in the evening compared to the morning in humans with obstructive sleep apnea. The mechanisms responsible for the diurnal variation in ventilatory long-term facilitation remain to be determined. However, one intriguing possibility is that evening to morning alterations in serotonin levels are responsible for our observations. Serotonin is a neuromodulator required for the initiation of ventilatory long-term facilitation in rats. Moreover, serotonergic levels vary between wakefulness and sleep, with levels being higher during the former state. More importantly, the synthesis and release of serotonin via adrenergic signaling in rats have been reported under constant lighting conditions, with peak

levels occurring later in the day and decreased synthesis and release in the early morning. Similar to the previous findings obtained during wakefulness we did observe differences in the magnitude of long-term facilitation of minute ventilation and tidal volume in the evening compared to the morning during sleep; although the magnitude of the difference was less during sleep compared to wakefulness. The reason for this discrepant finding remains to be determined. It is possible that the modulation of serotonin from the evening to the morning during sleep is not as great compared to the 'awake' state. Alternatively, it is possible that the modulation from evening to morning is significant enough to initiate differences but that the end-recovery time employed in the present investigation was not sufficient to allow clear differences in magnitude to manifest. More specifically it has been shown in animals including humans that long-term facilitation tends to "wind-up" during the recovery period following exposure to intermittent hypoxia. Typically this "wind-up" does not occur immediately at the onset of the recovery period. Consequently, because the end-recovery period was relatively short while the participants were treated with therapeutic continuous positive airway pressure clear differences in the magnitude of long-term facilitation between the evening and the morning might not have manifested. This is a possibility given that evening to morning differences appeared to manifest in measures of upper airway resistance (see below for additional details).

Impact of long-term facilitation on therapeutic positive airway pressure

As expected long-term facilitation of minute ventilation was accompanied by an increase in inspiratory flow during end recovery following exposure to intermittent hypoxia while the participants were treated with therapeutic continuous positive airway pressure. In contrast, airflow remained at baseline levels following exposure to the sham protocols. The increase in inspiratory flow could be the consequence of initiating long-term facilitation of upper airway and/or phrenic motoneurons. In regards to the testing of our primary hypothesis (see

Introduction) we were interested in establishing if long-term facilitation of upper airway muscle activity was initiated following exposure to intermittent hypoxia. To support this possibility we examined changes in upper airway resistance after exposure to intermittent hypoxia. Moreover, we determined if the upper airway was less collapsible by examining inspiratory airflow patterns following reductions in positive airway pressure. Our results showed that in addition to increases in inspiratory flow, upper airway resistance was reduced compared to baseline following exposure to intermittent hypoxia when the participants were treated with therapeutic continuous positive airway pressure. This finding is similar to the results reported by Rowley and colleagues. In contrast, upper airway resistance remained at baseline levels following exposure to the sham protocol. Moreover, when the positive airway pressure was reduced by 5 cmH_2O inspiratory airflow and resistance remained unaffected following exposure to intermittent hypoxia. These findings suggest that both upper airway collapsibility and resistance are impacted by exposure to mild intermittent hypoxia. Our results are in contrast to the findings of Rowley and colleagues who reported that upper airway collapsibility remained unaltered despite decreases in upper airway resistance following exposure to intermittent hypoxia (Rowley *et al.*, 2007). We also showed that inspiratory airflow was limited and upper airway resistance increased when the positive airway pressure was reduced by 5 cm H_2O following exposure to the sham protocol. Similarly although reductions in flow and increases in upper airway resistance were evident after the positive airway pressure was reduced by 7 cm H_2O following exposure to intermittent hypoxia the changes observed far exceeded the modifications in inspiratory flow and upper airway resistance when the positive airway pressure was reduced by a similar amount after exposure to the sham protocol.

The inspiratory flow modifications observed during end recovery in response to treatment with the therapeutic pressure or thereafter when the positive airway pressure was reduced were

similar in the evening compared to the morning. This findings suggests the magnitude of long-term facilitation of upper airway muscle activity or lack thereof (i.e. sham protocol) was not modulated by the time of day. Alternatively, increases in upper airway resistance were greater in the morning compared to the evening when the positive airway pressure was reduced by 5 and 7 cm H_2O following the sham protocol, indicating the possibility that long-term facilitation might be modulated by time of day.

The inspiratory airflow patterns observed following the sham protocol also indicated that time of day had no impact on upper airway collapsibility. This latter finding does not support our previous results which showed that collapsibility of the upper airway was greater in the morning compared to the evening. Likewise, it is counter to findings from the present study which showed that the therapeutic pressure required to eliminate apneic events was greater in the morning compared to the evening although admittedly the differences were not dramatic. Lastly, following the sham protocol upper airway resistance was greater in the morning compared to the evening after the positive airway pressure was reduced by 5 and 7 cm H_2O. Thus, the preponderance of evidence suggests that factors influencing upper airway resistance and collapsibility are likely impacted by the time of day.

Summary and conclusions

We have shown that exposure to mild intermittent hypoxia leads to the initiation of forms of respiratory plasticity that are beneficial to improving upper airway patency. More specifically we showed that following exposure to intermittent hypoxia continuous positive airway pressure could be reduced by 5 cm H_2O without impacting on inspiratory airflow and upper airway resistance. We propose that exposure to intermittent hypoxia might serve as an adjunct therapy to promote airway patency, which in turn could reduce the therapeutic pressure required to treat sleep apnea and potentially improve treatment compliance leading to improved outcome

measures. Likewise we propose that in addition to improving compliance mild intermittent hypoxia might have a direct impact on a number of co-morbid conditions associated with sleep apnea. Thus mild intermittent hypoxia could serve as a multi-pronged therapy to mitigate co-morbidities associated with sleep apnea both directly and indirectly by improving treatment compliance.

CHAPTER 6 - CONCLUSION

We have shown in the 2 aims discussed throughout this dissertation that multiple mechanisms involved in the control of breathing are indeed modulated in a circadian fashion, resulting in increased sensitivity to blood gas fluctuations, decreased carbon dioxide reserve and increased airway collapsibility. Those patterns were tightly correlated to with baseline carbon dioxide levels, potentially connecting them to a plethora of metabolic and hormonal changes. The subsequent breathing instability was evident in the observation that breathing events were longer in duration in N1 and N2 stages of sleep, and more frequent in the N1 stage. Such manifestation of the abovementioned patterns would have an impact on the treatment of obstructive sleep apnea with continuous positive airway pressure, as well as the recommended sleep scheduling and daytime naps. In addition, the therapeutic pressure required to eliminate one's breathing events will be largely affected. Titrations are therefore often overestimating the pressure for evening and afternoon sleep and/or underestimating it for morning sleep. Another therapeutic benefit from these findings is the identification of the increased collapsibility and chemoreflex sensitivity as potential pharmaceutical targets to mitigate the severity of OSA.

Given that a multitude of the discussed mechanisms is affected/modulated by serotonin, we then investigated a potential circadian variation of the initiation and magnitude of respiratory plasticity, in which serotonin is a key player. We were mainly interested in the long term facilitation of upper airway muscle activity. The focus on that form of plasticity was catalyzed by its importance in maintaining upper airway patency, and consequently affecting the TP.

Using intermittent hypoxia as a stimulus, we successfully initiated LTF of upper airway muscles, as evident by the ability of the airway to maintain optimal airflow at lower pressures, as well as a lack of resistance to that airflow. That effect was more maintainable in the evening compared to the morning (mainly in terms of developing resistance). Such findings can have a

big impact on OSA and CPAP therapy, one that has been largely marred by a low compliance rate due to uncomfortably high pressures. Initiating upper airway LTF and decreasing TP has the potential to revolutionize the approach to titration and CPAP prescription. IH, as an adjunct treatment, could be computerized and incorporated into the CPAP technology, which, along with the available auto-PAP abilities, might combine for a much more efficient and accurate way to administer positive pressure hopefully leading to a higher compliance level and consequently mitigating the severity of disorders caused by sleep apnea.

Finally, as we discussed in length in Chapter 5, IH in itself is garnering increasing support and evidence to its potential as a therapeutic modality, exerting both indirect and direct beneficial effects targeting many of the downstream co-morbidities of OSA, making it a multi-pronged therapeutic approach very worthy of further examination.

APPENDIX A

HIC IRB Approval Letters

WAYNE STATE UNIVERSITY

IRB Administration Office
87 East Canfield, Second Floor
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NOTICE OF FULL BOARD APPROVAL

To: Jason Mateika
Physiology
VA Medical Center

From: Lawrence R. Crane, M.D. or designee L. Crane, M.D. IM-Gr.
Chairman, Medical Institutional Review Board (M1)

Date: June 25, 2012

RE: IRB #: 060112M1F(V)
Protocol Title: Circadian Modulation of Breathing Stability and Respiratory Plasticity
Sponsor: ◦ NATIONAL INSTITUTES OF HEALTH
◦ VETERANS ADMINISTRATION
Protocol #: 1205010946

Expiration Date: June 06, 2013

Risk Level / Category: Research involving greater than minimal risk presenting no prospect of direct benefit, but likely to yield generalizable knowledge about the participant's condition

The above-referenced protocol and items listed below (if applicable) were **APPROVED** following *Full Board Review* by the Wayne State University Institutional Review Board (M1) for the period of 06/25/2012 through 06/06/2013. This approval does not replace any departmental or other approvals that may be required.

- The VA CIC recommends that the VA Electronic Medical Record not be flagged for this research and the IRB concurs. This is a sleep study that does not require the patient record to be flagged.
- The IRB has determined that all appropriate elements were included in the informed consent form, and are included in the informed consent process.
- Protocol (received in the IRB Office 5/8/12), and Protocol Summary Form (received in the IRB Office 5/8/12)
- HIPAA Summary Form (received in the IRB Office 5/8/12) and Authorization for Release of Indentifiable Health Information for Research Purposes
- VA Research Pre-Screening Form (dated 02/12)
- VA Research Consent Form - Study 1
- VA Research Consent Form - Study 2
- VA Research Consent Form - Study 3
- Subject Phone Interview Form
- Screen Failure Report
- Participant Materials: Notice of Privacy Practices, Notice of Privacy Practices Acknowledgement, and Non-Veteran Research Form
- Advertisements: List of places for advertisements, John D. Dingell VA Medical Center Advertisement, Wayne State University and John D. Dingell VA Medical Center Advertisement, Metro Times Newspaper Advertisement, Craigslist Advertisement, Facebook Advertisement, and 2 additional flyers
- Data Collection Tools: Subject Personal Information Sheet, Physical Examination Form, Stanford Sleepiness Scale, and Epworth Sleepiness Scale

◦ Federal regulations require that all research be reviewed at least annually. You *may* receive a *"Continuation Renewal Reminder"* approximately two months prior to the expiration date; however, it is the Principal Investigator's responsibility to obtain review and continued approval **before** the expiration date. Data collected during a period of lapsed approval is unapproved research and can *never* be reported or published as research data.

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NOTICE OF FULL BOARD CONTINUATION APPROVAL

To: Jason Mateika
Physiology
VA Medical Center

From: Lawrence R. Crane, M.D. or designee
Chairman, Medical Institutional Review Board (M1)

Date: April 03, 2014

RE:
IRB #: 060112M1F(V)
Protocol Title: Circadian Modulation of Breathing Stability and Respiratory Plasticity
Funding Source: Sponsor: NATIONAL INSTITUTES OF HEALTH
Sponsor: VETERANS ADMINISTRATION
Protocol #: 1205010946

Expiration Date: April 02, 2015

Risk Level / Category: Research involving greater than minimal risk presenting no prospect of direct benefit, but likely to yield generalizable knowledge about the participant's condition

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Continuation for the above-referenced protocol and items listed below (if applicable) were **APPROVED** following Full Board review by the Wayne State University Institutional Review Board (M1) for the period of 04/03/2014 through 04/02/2015. This approval does not replace any departmental or other approvals that may be required.

- Actively accruing participants
- 1) VA Research Pre-Screening Form (dated 9/4/12), 2) VA Research Consent Form (Study 1) (dated 8/4/12), 3) VA Research Consent Form (Study 2) (dated 9/4/12), and VA Research Consent Form (Study 3) (dated 9/4/12)
- Screen Failure Report
- Advertisement Text (4) and Flyers (3)

- Federal regulations require that all research be reviewed at least annually. You *may* receive a *"Continuation Renewal Reminder"* approximately two months prior to the expiration date; however, it is the Principal Investigator's responsibility to obtain review and continued approval ***before*** the expiration date. Data collected during a period of lapsed approval is unapproved research and can *never* be reported or published as research data.
- All changes or amendments to the above-referenced protocol require review and approval by the IRB **BEFORE** implementation.
- Adverse Reactions/Unexpected Events (AR/UE) must be submitted on the appropriate form within the timeframe specified in the IRB Administration Office Policy (http://www.irb.wayne.edu/policies-human-research.php).

NOTE:

1. Upon notification of an impending regulatory site visit, hold notification, and/or external audit the IRB Administration Office must be contacted immediately.
2. Forms should be downloaded from the IRB website at **each** use.

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NOTICE OF FULL BOARD CONTINUATION APPROVAL

To: Jason Mateika
Physiology
VA Medical Center

From: Lawrence R. Crane, M.D. or designee L. Crane MD/MB
Chairman, Medical Institutional Review Board (M1)

Date: March 05, 2015

RE:

IRB #:	060112M1F(V)
Protocol Title:	Circadian Modulation of Breathing Stability and Respiratory Plasticity
Funding Source:	Sponsor: NATIONAL INSTITUTES OF HEALTH Sponsor: VETERANS ADMINISTRATION
Protocol #:	1205010946
Expiration Date:	March 04, 2016
Risk Level / Category:	Research involving greater than minimal risk presenting no prospect of direct benefit, but likely to yield generalizable knowledge about the participant's condition

Continuation for the above-referenced protocol and items listed below (if applicable) were **APPROVED** following Full Board review by the Wayne State University Institutional Review Board (M1) for the period of 03/05/2015 through 03/04/2016. This approval does not replace any departmental or other approvals that may be required.

- Actively accruing participants
- VA Research Pre-Screening Form (dated 9/4/12)
- VA Research Consent Form Study 1 (dated 9/4/12)
- VA Research Consent Form Study 2 (dated 9/4/12)
- VA Research Consent Form Study 3 (dated 9/4/12)
- Authorization for Release of Identifiable Health Information For Research Purposes
- Screen Failure Report
- Advertisements (7)

- Federal regulations require that all research be reviewed at least annually. You *may* receive a *"Continuation Renewal Reminder"* approximately two months prior to the expiration date; however, it is the Principal Investigator's responsibility to obtain review and continued approval ***before*** the expiration date. Data collected during a period of lapsed approval is unapproved research and can *never* be reported or published as research data.
- All changes or amendments to the above-referenced protocol require review and approval by the IRB **BEFORE** implementation.
- Adverse Reactions/Unexpected Events (AR/UE) must be submitted on the appropriate form within the timeframe specified in the IRB Administration Office Policy (http://www.irb.wayne.edu/policies-human-research.php).

NOTE:

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2. Forms should be downloaded from the IRB website at **each** use.

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NOTICE OF FULL BOARD CONTINUATION APPROVAL

To: Jason Mateika
Physiology
VA Medical Center

From: Lawrence R. Crane, M.D. or designee L. Crane / CB
Chairman, Medical Institutional Review Board (M1)

Date: February 04, 2016

RE:

IRB #:	060112M1F(V)
Protocol Title:	Circadian Modulation of Breathing Stability and Respiratory Plasticity
Funding Source:	Sponsor: NATIONAL INSTITUTES OF HEALTH Sponsor: VETERANS ADMINISTRATION
Protocol #:	1205010946
Expiration Date:	February 03, 2017
Risk Level / Category:	Research involving greater than minimal risk presenting no prospect of direct benefit, but likely to yield generalizable knowledge about the participant's condition

Continuation for the above-referenced protocol and items listed below (if applicable) were **APPROVED** following Full Board review by the Wayne State University Institutional Review Board (M1) for the period of 02/04/2016 through 02/03/2017. This approval does not replace any departmental or other approvals that may be required.

- Actively accruing participants.
- VA Research Pre-Screening Form (dated 9/4/12)
- VA Research Consent Form Study 1 (dated 9/4/12)
- VA Research Consent Form Study 2 (dated 9/4/12)
- VA Research Consent Form Study 3 (dated 9/4/12)
- Authorization for Release of Identifiable Health Information For Research Purposes
- Screen Failure Report
- Advertisements (7)

- Federal regulations require that all research be reviewed at least annually. You *may* receive a *"Continuation Renewal Reminder"* approximately two months prior to the expiration date; however, it is the Principal Investigator's responsibility to obtain review and continued approval ***before*** the expiration date. Data collected during a period of lapsed approval is unapproved research and can *never* be reported or published as research data.
- All changes or amendments to the above-referenced protocol require review and approval by the IRB **BEFORE** implementation.
- Adverse Reactions/Unexpected Events (AR/UE) must be submitted on the appropriate form within the timeframe specified in the IRB Administration Office Policy (http://www.irb.wayne.edu/policies-human-research.php).

NOTE:

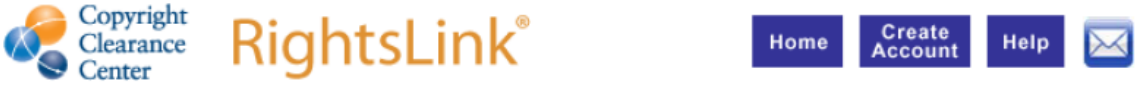
1. Upon notification of an impending regulatory site visit, hold notification, and/or external audit the IRB Administration Office must be contacted immediately.
2. Forms should be downloaded from the IRB website at **each** use.

APPENDIX B

Copyright License Agreement for Chapter 2

APPENDIX C

Copyright License Agreement for Chapter 3



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

Author: Mohamad El-Chami,David Shaheen,Blake Ivers,Ziauddin Syed,M. Safwan Badr,Ho-Sheng Lin,Jason H. Mateika

Publication: Journal of Applied Physiology

Publisher: The American Physiological Society

Date: Nov 15, 2014

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ABSTRACT

CIRCADIAN MODULATION OF BREATHING STABILITY AND RESPIRATORY PLASTICITY

by

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May 2017

Advisor: Jason Mateika, Ph.D.

Major: Physiology

Degree: Doctor of Philosophy

Purpose: Our project was designed to determine the effect of time of day on multiple mechanisms influencing breathing stability and respiratory plasticity. We investigated if the number and duration of breathing events coupled to upper airway collapsibility, as well as the carbon dioxide reserve, chemoreflex sensitivity and arousal threshold during non-rapid eye movement (NREM) sleep were affected by the time of day. In addition, we examined if mild intermittent hypoxia (IH) initiates long-term facilitation of upper airway muscle activity leading to a reduction in the therapeutic continuous positive airway pressure required to eliminate breathing events.

Methods: 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. For breathing events detected during these sessions the rate of change of respiratory effort, maximum respiratory effort immediately prior to termination of an event, and the maximum tidal volume and the minimum partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) immediately following an event were measured. Participants then completed

the same protocol on two additional occasions, where the critical closing pressure that demarcated upper airway collapsibility was determined on one, and baseline levels of carbon dioxide $P_{ET}(CO_2)$ and minute ventilation, as well as the $P_{ET}(CO_2)$ that demarcated the apneic threshold and hypocapnic ventilatory response were measured on the other (the order of these 2 visits was randomized). In the second aim of the study, male participants with obstructive sleep apnea were treated with twelve 2-minute episodes of hypoxia ($P_{ET}O_2 \approx 50$ mmHg) separated by 2-minute intervals of normoxia in the presence of $P_{ET}CO_2$ that was sustained 3 mmHg above baseline. During recovery from the last episode the positive airway pressure was reduced in a step-wise fashion until flow limitation was evident. The participants also completed a sham protocol under normocapnic conditions, which mimicked the timeframe of the IH protocol.

Results: 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 cm H_2O) compared with the evening (1.29 ± 0.91 cm H_2O; $P \leq 0.02$) and afternoon (1.25 ± 0.79; $P \leq 0.01$). 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 ($r = -0.73$, $P \leq 0.005$). The nadir of core body temperature during sleep occurred in the morning and was accompanied by reductions in minute ventilation and $P_{ET}CO_2$ compared with the evening and afternoon (minute ventilation: 5.3 ± 0.3 vs. 6.2 ± 0.2 vs. 6.1 ± 0.2 l/min, $P < 0.02$; $P_{ET}(CO_2)$: 39.7 ± 0.4 vs. 41.4 ± 0.6 vs. 40.4 ± 0.6 Torr, $P < 0.02$). The carbon dioxide reserve was reduced, and the hypocapnic ventilatory response increased in the morning compared with the evening and afternoon (carbon dioxide reserve: 2.1 ± 0.3 vs. 3.6 ± 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 l·min(-1)·mmHg(-1), $P < 0.001$). The rate of change of respiratory effort was similar in N2 compared to N1 but the

maximum respiratory effort immediately prior to event termination was greater (-10.7 ± 1.2 vs. -9.6 ± 1.0 cm H2O/s, $P < 0.05$). Likewise, tidal volume was increased (1169 ± 105 vs. 1082 ± 100 ml, $P < 0.05$) and PETCO2 was decreased (37.0 ± 0.8 vs. 37.7 ± 0.8 mmHg $P < 0.05$) following events in N2 compared to N1. A similar tidal volume and PETCO2 response was evident following events in the morning compared to the evening independent of sleep stage. After exposure to IH the therapeutic pressure was significantly reduced (Δ CPAP = - 4.95 ± 0.5 cmH2O, $p < 0.001$) without evidence of flow limitation ($P > 0.2$) or increases in upper airway resistance ($P > 0.4$). In contrast, a similar decrease in pressure was accompanied by significant flow limitation ($P < 0.003$) and an increase in upper airway resistance ($P < 0.01$) following completion of the sham protocol.

Conclusion: Our findings indicate that time of day affects the duration and frequency of events, coupled with alterations in upper airway collapsibility and 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. 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, which are independent of the consequences of sleep apnea. We also conclude that alterations in the arousal threshold, reflected by an increase in respiratory effort at event termination, coupled to increases in tidal volume and reductions in $P_{ET}CO_2$ contribute to modifications in event duration and frequency associated with variations in sleep state or time of night. In addition, exposure to IH decreases the therapeutic pressure required to eliminate apneic events which could improve treatment compliance. This possibility coupled with the direct beneficial effects of IH on co-morbidities linked to sleep apnea suggests that IH may have a multipronged therapeutic effect on sleep apnea.

AUTOBIOGRAPHICAL STATEMENT

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Education

2003-08 B.S. in Biology (with honors) Lebanese University
2008-11 M.Sc.) in Physiology, American University of Beirut, School of Medicine
2012- Ph.D. in Physiology, Wayne State University, School of Medicine

Awards and Honors

- Recipient of the Centennial Award for Outstanding Achievement in the Physiology Graduate Program 2016
- Recipient of the "Rumble Fellowship" for the academic year 2013-2014, department of physiology, Wayne State University, School of Medicine.
- Recipient of the Marion I. Barnhart Graduate Student Award for the academic year 2013-2014 (received in August 2014)
- First place winner in the oral presentations category - 2014 graduate student research day
- Recipient of the Department of Physiology travel award to present an abstract at the 2015 American Thoracic Society (ATS) international conference (May 2015 - Denver)
- Recipient of the WSU graduate school travel award to present an abstract at the 2015 American Thoracic Society (ATS) international conference (May 2015 - Denver)
- Recipient of the American Thoracic Society to present an abstract at the 2015 American Thoracic Society (ATS) international conference (May 2015 - Denver)
- Recipient of the I. Robin Barraco Memorial Scholarship award for the academic year 2014-2015 (received in August 2015)
- Award for best poster presentation - Michigan Physiological Society (MPS) conference (May 2016)

Publications

1. **El Chami M**, Shaheen D, Ivers B, Syed Z, Badr MS, Lin HS, Mateika JH. "Time of day affects chemoreflex sensitivity and the carbon dioxide reserve during NREM sleep in participants with sleep apnea". *Journal of Applied Physiology*. 2014 Nov 15;117(10):1149-56.
2. Mateika, J. H., **El-Chami M**., Shaheen, D., & Ivers, B. (2014). "Intermittent hypoxia: A low risk research tool with therapeutic value in humans". *Journal of Applied Physiology*, 2015 Mar 1;118(5):520-32.
3. **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". *Journal of Applied Physiology* (June 2015).
4. Wains S, **El-Chami M**, Lin HS and Mateika JH. "Impact of arousal threshold and respiratory effort on the duration of breathing events across sleep stage and time of night". *Respiratory Physiology & Neurobiology* (December 2016)
5. **El-Chami M**, Sudan S, Shaheen DJ, Jassim A, Lin HS and Mateika JH. "Mild Intermittent Hypoxia with Sustained Hypercapnia Reduces Therapeutic CPAP and Improves Airflow in Participants with Obstructive Sleep Apnea". ***Completed***, to be submitted in the upcoming few weeks.
6. **El-Chami M**, Sudan S, Lin HS and Mateika JH. "Sigmoidal upper airway pressure-flow relationship in individuals with obstructive sleep apnea". ***In preparation***, to be submitted in the upcoming few weeks.