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Original Article

ORIGINAL ARTICLE

Sleep-disordered breathing in C57BL/6J mice with diet-induced obesity

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Abstract

Obesity leads to sleep-disordered breathing (SDB) manifested by recurrent upper airway obstructions termed obstructive sleep apnea (OSA) and carbon dioxide retention due to hypoventilation. The objective of this work was to characterize breathing during sleep in C57BL6/J mice with diet-induced obesity (DIO). Arterial blood gas was measured in nine obese and nine lean mice during wakefulness. Nine male mice with DIO and six lean male C57BL/6J mice were head mounted with electroencephalogram (EEG) and electromyogram (EMG) electrodes. Sleep recordings were performed in the whole body plethysmography chamber; upper airway obstruction was characterized by the presence of inspiratory flow limitation in which airflow plateaus with increases in inspiratory effort. Obese mice showed significantly lower pH and higher partial pressure of arterial CO_2 ($PaCO_2$) in arterial blood gas compared to lean mice, 7.35 ± 0.04 versus 7.46 ± 0.06 ($p < 0.001$) and 38 ± 8 mm Hg versus 30 ± 5 mm Hg ($p < 0.001$). Obese mice had similar levels of minute ventilation to lean mice during sleep and wakefulness, despite higher body weight and temperature, indicating an increase in the metabolic rate and hypoventilation. Obese mice also showed baseline hypoxemia with decreased mean oxyhemoglobin saturation across sleep/wake states. Obese mice had a higher prevalence of flow-limited breathing compared to lean mice during sleep. However, the oxygen desaturation index in lean and obese mice did not differ. We conclude that DIO in mice leads to hypoventilation. Obesity also increases the frequency of inspiratory limited breaths, but it does not translate into progression of OSA.

Statement of Significance

Obesity hypoventilation and obstructive sleep apnea (OSA) are complications of obesity. Breathing during sleep in rodents with diet-induced obesity (DIO) has not been previously examined. In this article, we have shown that diet-induced obese mice develop obesity hypoventilation with significant increases in CO_2 and decreases in pH during wakefulness and hypoxemia across sleep/wake states. Obese mice had inspiratory flow limitation during non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep, but recurrent pharyngeal obstruction with oxyhemoglobin desaturations similar to human OSA was not present. We conclude that DIO in mice leads to hypoventilation without overt OSA. Our data will facilitate future studies of the pathogenesis of sleep-disordered breathing in obesity.

Key words: sleep apnea; obesity hypoventilation; mouse model; leptin

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Introduction

Obesity is associated with a spectrum of sleep-disordered breathing (SDB) including periodic apneas and hypopneas as well as sustained nocturnal disturbances in gas exchange. Obstructive sleep apnea (OSA) is characterized by recurrent periods of upper airway obstruction (apneas and hypopneas) during sleep, leading to nocturnal hypercapnia, repeated oxyhemoglobin desaturations and arousals [1]. The prevalence of OSA is 24%–27% in middle age men and 40%–45% in older men, 9% in middle age women and 25%–30% in older women [2], but it exceeds 50% in obese individuals [2, 3]. OSA is a major cause of morbidity and mortality in Western society [4, 5] and contributes significantly to the development and progression of neurocognitive, metabolic, cardiovascular, and oncologic diseases [6–10]. Nasal continuous positive airway pressure relieves upper airway obstruction during sleep, but it has poor adherence [11].

In addition to OSA, obesity is associated with alveolar hypoventilation during sleep identified by sustained O_2 desaturation and increase in transcutaneous CO_2 levels during sleep in the absence of apneas and hypopneas [12]. Hypoventilation can result from prolonged periods of respiratory depression and/or sustained upper airway obstruction during sleep, which can be present apart from apneic and hypopneic events in patients with OSA or in patients without overt OSA [12]. Central hypoventilation during sleep in obesity is caused by the failure of the respiratory pump muscles to respond to the CO_2 load due to impaired respiratory mechanics or faulty control of breathing, and the latter being considered a predominant mechanism [13]. An adipose-tissue produced hormone leptin is a potent respiratory stimulant [14–17], and leptin resistance has been implicated in the pathogenesis of sleep-related hypoventilation in obesity [18]. Obesity hypoventilation syndrome (OHS) is defined as daytime hypercapnia in an obese patient with SDB in the absence of any other causes of hypoventilation [19]. OHS is observed in 10%–20% obese patients with OSA and 0.15%–0.3% of the adult population. OHS leads to pulmonary hypertension and congestive heart failure with ~30% 5-year mortality [19]. Non-invasive ventilation remains the most effective treatment of OHS.

Therapeutic development has been hindered by the lack of high throughput animal models, which would mimic human obesity-related SDB phenotypes. In the Yucatan miniature pig [20] OSA is present in both non-rapid eye movement (NREM) and rapid eye movement (REM) sleep only in the presence of obesity, but this non-rodent model does not allow high throughput studies. Brennick et al. [21] have shown that New Zealand Obese mice have narrow pharyngeal airway and the upper airway patency in these mice depended on inspiratory activation [22], similar to human OSA. However, these studies were based on magnetic resonance imaging under anesthesia, while sleep was not recorded. We had previously shown that leptin-deficient obese *ob/ob* mice have both OHS with depressed hypercapnic ventilatory response and increased awake partial pressure of arterial CO_2 ($PaCO_2$) [15], and OSA, predominantly in REM sleep [17, 23] and that both defects were abolished by leptin treatment. A major shortcoming of the *ob/ob* mouse model is that respiratory abnormalities are associated with leptin deficiency, which is uncommon in obese humans [24].

The goal of the present study was to examine effects of diet-induced obesity (DIO) on sleep and breathing in C57BL/6J mice. We hypothesized that mice with DIO will have SDB manifested as upper airway obstruction during sleep similar to human OSA and obesity hypoventilation.

Methods

Animals

Thirty-three adult male C57BL/6J mice, 6–8 weeks of age (Jackson Laboratory, Bar Harbor, MA) were fed with a high fat diet (TD 03584, Teklad WI, 5.4 kcal/g, 35.2% fat, 58.4% kcal from fat, $n = 18$) or chow diet (3.0 kcal/g, 4.4% fat, 13% kcal from fat, $n = 15$) for 14 weeks. Food and water were provided ad libitum. Mice were housed in a standard laboratory environment at 22°C in the 12 hours light/dark cycle (09:00 am–09:00 pm lights on/09:00 pm–09:00 am lights off). Fifteen mice (nine DIO mice and six lean mice) were used for sleep recordings. Eighteen mice (nine DIO mice and nine lean mice) were used for arterial blood gas measurements. All surgical procedures were performed under 1%–2% isoflurane anesthesia. Burpenorphine (0.01 mg/kg) was administered subcutaneously at the end of surgery to minimize discomfort. The study was approved by the Johns Hopkins University Animal Use and Care Committee (Protocol # MO16M161) and complied with the American Physiological Society Guidelines for Animal Studies.

Electroencephalogram/ electromyogram electrode implantation

Electroencephalogram (EEG) and electromyogram (EMG) electrodes were implanted with an EEG/EMG Headmount (Pinnacle Technology, Lawrence, KS) as previously described [25]. Briefly, four electroencephalographic (EEG) Teflon-coated stainless steel wire electrodes were inserted into the skull through predrilled holes and bonded to the dorsal surface of the skull with dental acrylic (Land Dental, Wheeling, IL) under 1%–2% isoflurane anesthesia. Two nuchal electromyographic (EMG) electrodes were tunneled subcutaneously and placed over the nuchal muscles posterior to the skull. Mice were allowed to recover for 3–4 days prior to polysomnography.

Mouse polysomnography

Whole body plethysmography (WBP) recordings (mouse whole body plethysmograph, Buxco, Wilmington, NC) were performed as previously described [25]. In brief, the animals were placed in a modified WBP open-system chamber to allow for prolonged ~6-hour recordings (10:00 am–04:00 pm) [25]. The WBP chamber had the internal diameter of 80 mm, the height of 50.5 mm and the volume approximately 0.4 L. The chamber was equipped with two ports (pneumotachographs) on the upper surface and one large-side port and three small-side ports at the base, which we utilized to customize our system and optimize its performance. Positive and negative pressure sources were utilized in series with mass flow controllers and high-resistance elements to generate a continuous bias flow through the animal chamber while maintaining a sufficiently high time constant. The reference chamber serves to filter ambient noise

from the pressure signal. Slow leaks present on both chambers allowed for equilibration with atmospheric pressure. A respiratory effort sensor bladder was placed under the mouse to transduce the mechanical pressure changes associated with mouse breathing, while the reference bladder signal allowed for cancellation of the contaminating chamber pressure signal via the differential pressure transducer. The Drorbaugh and Fenn equation was used to calculate the WBP tidal volume (V_T) signal from the WBP chamber pressure signal [26]. Application of this formula required the measurement of the following variables during each WBP recording session: mouse rectal temperature, chamber temperature, room temperature and relative humidity, and chamber gas constant, which was calculated by utilizing a known volume injection and the resultant WBP pressure deflection.

Mice were habituated to the WBP chamber from 10:00 am to 04:00 pm one day before the recording session. During full polysomnographic recording sessions, the chamber was humidified to 90% relative humidity, and the mouse was allowed 45 minutes to acclimate to the chamber before recording. All signals were digitized at 1000 Hz (sampling frequency per channel) and recorded in LabChart 7 Pro (Version 7.2, ADInstruments, Dunedin, NZ) [27]. Respiratory signals were analyzed from all REM sleep periods and from periods of NREM sleep sampled periodically at 20-second stretches every half an hour throughout the total recording time. NREM stretches were selected from NREM periods of at least 1 minute. Custom software was used to demarcate the start and end of inspiration and expiration for subsequent calculations of timing and amplitude parameters for each respiratory cycle. Oxyhemoglobin saturation (SpO_2) was monitored using pulse oximetry with a mouse neck collar clip (Starr Life Science, Oakmont, PA). Body temperature was measured by a rectal probe. Additional customized software had been developed to automate our ability to demarcate each inspiration, classify the breath as flow limited and describe maximal inspiratory airflow (V_{Imax}) and components of minute ventilation (V_E).

Data analysis

Studies were scored by two independent scorers (CCE and HP), who were blinded to study conditions. Sleep-wake state was scored visually in 5-second epochs from 10:00 am until 04:00 pm. Standard criteria were employed to score sleep-wake state based on EEG and EMG frequency content and amplitude, as previously described [28]. Wakefulness was characterized by low-amplitude, high-frequency (~10 to 20 Hz) EEG waves and high levels of EMG activity compared with the sleep states. NREM sleep was characterized by high-amplitude, low frequency (~2 to 5 Hz) EEG waves with EMG activity considerably less than during wakefulness. REM sleep was characterized by low-amplitude, mixed frequency (~5 to 10 Hz) EEG waves with EMG amplitude either below or equal to that during NREM sleep.

Respiratory analysis

The instantaneous respiratory rate (RR, breaths/min) was calculated as the reciprocal of the respiratory period, and the instantaneous V_E (mL/min) was product of the RR and V_T for each breath. We then utilized the airflow and respiratory effort signals to develop an algorithm for detecting upper airway obstruction during sleep. Obstruction was characterized by the development of inspiratory flow limitation (IFL) in the presence of increased effort. Once breaths were deemed to be flow-limited, we defined the severity of airflow obstruction by the absolute peak level of V_{Imax} [30].

The algorithm for detecting IFL was developed based on airflow timing and amplitude parameters for each inspiration as previously described [29] and outlined in Supplementary Figure as follows. Custom software provided a discrete value for inspiratory flow (V_I) at the inspiratory midpoint (V_{I50}). This midpoint was then used to partition inspiration into early and late phases. Peak inspiratory flow during the early and late phases were defined as V_{Imax1} and V_{Imax2}, respectively. Breaths were considered to be non-flow limited when a plateau in mid-inspiratory flow could not be discerned. These breaths (sniffs) were defined by their short duration when the total inspiratory time was less than 1.75*SD of the mean inspiratory time of the entire sample of breaths, and were excluded.

For the remaining inspirations with durations greater than 1.75*SD, IFL was defined if a plateau in mid-inspiratory flow was detected, as follows [1]. Breaths with an early peak of inspiratory flow had to be followed by a plateau of sufficient duration. A plateau was considered to be present if V_{Imax1} was greater than both V_{I50} and V_{Imax2} (decrescendo pattern), and if the time between the early and late flow peaks were ≥ 20% of the total inspiratory time (Supplementary Figure, Arm A). At times, a decrease in flow could be discerned beyond the point of V_{Imax1}, consistent with the phenomenon of negative effort dependence in IFL breaths.

Alternatively, IFL was defined for breaths in which the late inspiratory peak in airflow exceeded the early peak. Under these circumstances, IFL was defined by the presence of an inward convexity in the mid-inspiratory airflow signal (flanking V_{I50}, see arm B in Supplementary Figure). The pattern was present when the early inspiratory peak was considerably greater than a threshold, given by the level of V_{I50} + the *SD* of the V_{Imax1}, V_{Imax2}, and V_{I50}.

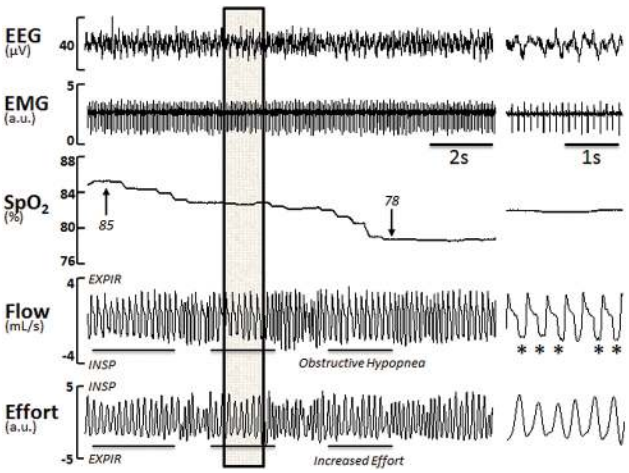

Figure 1. A representative trace of REM sleep in the diet-induced obese mouse. Left panel, shows compressed recording of EEG, nuchal EMG, pulse oximetry (SpO_2), respiratory flow and effort. Obstructive hypopneas characterized by inspiratory flow limitation with increases in respiratory effort terminated by oxygen desaturations (see the arrows). Right panel, the shaded area on the left panel is decompressed. The asterisks indicate flow limited breaths. *EXPIR* denotes expiration and *INSP* denotes inspiration.

Table 1. Basic characteristics of lean and diet-induced obese C57BL/6J mice, which underwent sleep studies

Phenotype	N	Age (weeks)	Body weight (g)	Body temperature (°C)	Plasma Leptin (ng/mL)
Lean	6	19.1 ± 1.5	28.1 ± 1.3	35.1 ± 0.1	1.4 ± 0.4
Obese	9	21.4 ± 1.6	44.7 ± 1.8*	36.1 ± 0.1*	47.9 ± 7.5**

Values are presented as means ± *SEM*.
*$p < 0.01$; ** $p < 0.001$.

The oxygen desaturation index (ODI) was defined as a number of oxyhemoglobin desaturations ≥3% from the baseline (for at least two breaths) per hour of sleep. T90 was defined as a percentage of recording time spent with SpO_2 below 90%. A representative recording of flow-limited breathing leading to recurrent obstructive hypopneas with oxyhemoglobin desaturations during REM sleep in the DIO mouse is presented in Figure 1. A distinct mid-inspiratory flow plateau was accompanied by increased effort during the hypopneas, indicating the presence of inspiratory airflow limitation with upper airway obstruction.

Arterial blood gases experiment

An arterial catheter was implanted in the left femoral artery under 1%–2% isoflurane anesthesia. The femoral artery was carefully exposed via 0.5 cm incision and an arterial catheter was inserted 5–8 mm deep into the femoral artery, glued in place and routed under the skin. The skin was sutured with 6.0 silicon-coated silk sutures. The catheter was attached to a single-channel fluid swivel (model 375/25, Instech Laboratories) and perfused slowly by an infusion pump (0.5 mL/day) with a sterile saline solution containing heparin (1,000 U heparin/L saline). Mice were recovered for a minimum of 48 hours prior to blood gas testing, which was performed in awake unrestrained mice removing 150 uL of arterial blood, which was processed in a blood analyzer (Radiometer ABL 800 Flex, Diamond Diagnostics, Holliston, MA).

Plasma leptin

At the end of the experiment, a terminal blood draw was performed from the arterial line or inferior vena cava under 1%–2% isoflurane anesthesia. Plasma leptin was measured with an ELISA kit (Millipore, Burlington, MA).

Statistical analyses

The primary objective of the present study was to examine effects of DIO on SDB. Our primary outcome was the percentage of flow-limited breaths during sleep. Our secondary outcomes were severity of disturbances in ventilation and oxygenation. The statistical analysis was designed to examine effects of DIO on measures of upper airway obstruction, ventilation, and gas exchange disturbances. Specifically, upper airway obstruction severity was characterized by the percentage of breaths that exhibited IFL and the level of V_{Imax} during flow limited breathing. Ventilation was assessed by V_E and its components, V_T and RR, during non-flow limited and flow limited breathing. The severity of gas exchange abnormalities was characterized by T90 and mean SpO_2 across sleep/wake states. The periodicity of gas exchange abnormalities (measures of sleep apnea) was assessed by ODI. SDB parameters were compared between groups (DIO vs. lean mice) with the Wilcoxon rank-sum tests (STATA 12, StataCorp LP, College Station TX). Because sleep stage can markedly affect SDB severity, we stratified analyses by NREM versus REM sleep. In all cases, a p-value < 0.05 was considered significant.

We powered our study to detect an increase in percentage of IFL breaths in DIO mice compared to lean mice of 10%, with a within-group standard deviation of 5%. Under these assumptions, we calculated a standardized effect size of 2. We, therefore, estimated that six mice in each group would result in a 90% chance to detect a difference in the IFL prevalence.

Results

Baseline characteristics

DIO mice were 16.6 g heavier than lean mice (Table 1). There was no statistically significant difference in age between two groups. The rectal body temperature was 1°C higher in DIO mice than in lean mice suggesting a higher metabolic rate. As expected, DIO mice had a greater than 30-fold increase in plasma leptin levels compared to lean mice with large variations between the animals. The arterial blood gas in awake DIO mice showed a significant increase in $PaCO_2$ and a decrease in pH ($p < 0.001$ for both, Table 2 and Figure 2), compared to lean mice, which demonstrates that DIO mice hypoventilated. A difference in PaO_2 between two phenotypes did not reach statistical significance (Table 2).

Polysomnography revealed a significant decrease in sleep efficiency and the amount of NREM sleep in DIO mice due to a decrease in a number of NREM sleep bouts, whereas bout duration remained the same as in lean mice (Table 3). The amount of REM sleep varied within dietary groups without a significant difference between the groups.

Table 2. Arterial blood gas in awake unrestrained lean and obese C57BL/6J mice

Phenotype	N	Body weight	pH	$PaCO_2$ (mm Hg)	PaO_2 (mm Hg)
Lean	9	28.4 ± 5.3	7.46 ± 0.06	30 ± 5	98 ± 3.9
Obese	9	45.1 ± 5.3*	7.35 ± 0.04**	38 ± 8**	91.9 ± 1.7

Values are presented as medians ± interquartile range.
*$p < 0.01$; **$p < 0.001$.

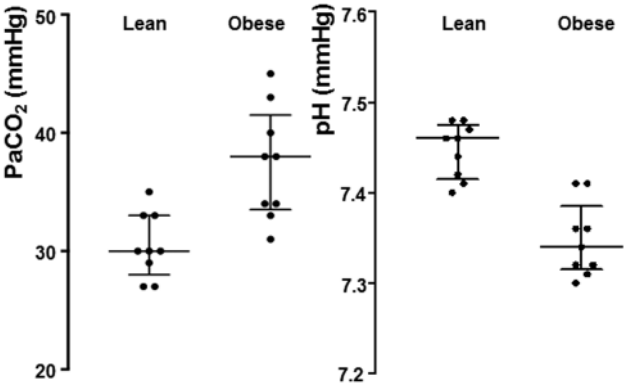

Figure 2. Partial pressure of carbon dioxide ($PaCO_2$) and pH of the arterial blood obtained from awake unrestrained lean and obese C57BL/6J mice. Values per individual mice are depicted as black circles. Central horizontal lines denote the medians, top and bottom horizontal lines represent the second and third quartiles, respectively. $p < 0.001$ for differences between two phenotypes for both $PaCO_2$ and pH.

Obesity, ventilation, and oxygenation during sleep

Non-flow limited breathing and flow limited breathing metrics characterize control of breathing and upper airway function during sleep respectively and therefore were analyzed separately. DIO and lean mice demonstrated the same level of ventilation during non-flow limited breathing awake and throughout sleep stages, despite a large difference in body weight (Figure 3A). V_T and RR (Figure 3, B and C) were also similar, suggesting hypoventilation in DIO compared to lean mice.

DIO mice showed a marked increase in time spent with $SpO_2 < 90\%$ (70 ± 2% of total sleep time vs. 45 ± 2% in lean mice, $p < 0.01$, Figure 4A). DIO lowered mean SpO_2 during wakefulness (Figure 4B), but demonstrated even greater reductions of mean SpO_2 during NREM sleep (91% ± 1% vs. 95% ± 1% in lean mice, $p < 0.05$). During REM sleep SpO_2 also trended to be lower in DIO mice (84% ± 3% vs. 91% ± 2% in lean mice, $p = 0.06$), but the difference between the phenotypes did not reach statistical significance.

Obesity and sleep apnea

Flow limited breaths were sporadic during wakefulness and, therefore, were not quantified. DIO mice demonstrated a significant increase in the percentage of flow limited breaths in NREM sleep (14.9% ± 5% vs. 2.2% ± 1.2% in lean mice, $p < 0.05$). The difference in percentage of flow limited breaths in REM sleep did not reach statistical significance (43.8% ± 9.0% vs. 22.5% ± 4.3% in lean mice, $p = 0.07$, Figure 5A). There was no difference in V_E, V_T, RR, or V_{Imax} during flow limited breathing between lean and obese animals (Figure 5, B–E). The ODI was significantly higher in REM sleep compared to NREM sleep in both groups of mice, regardless of the diet ($p < 0.01$). There was no significant difference in ODI between lean and obese mice during NREM and REM sleep (Figure 6).

Discussion

To our knowledge, this is the first study examining breathing during sleep in diet-induced obese mice. The main finding of our study was that DIO in mice leads to hypoventilation during wakefulness and sleep. Specifically, DIO mice showed decreased pH and increased CO_2 in the arterial blood during wakefulness and the same levels of V_E across sleep/wake stages as lean mice, despite higher body weight and body temperature. These findings suggest that DIO mice hypoventilated, which accounted for reduced mean SpO_2 across sleep/wake states. In addition, DIO induced IFL both during NREM and REM sleep, which could contribute to hypoventilation. The prevalence of sleep apnea, however, was not increased compared to lean mice as was evident from similar indices of periodic breathing, that is, ODI.

DIO mice and hypoventilation during sleep

DIO mice were significantly more hypoxemic across sleep/wake stages compared to lean mice (Figure 4). Hypoxemia can be related to shunt, ventilation-perfusion mismatch, and hypoventilation. Our data demonstrate that DIO mice had higher CO_2 levels and lower pH in the arterial blood gas than lean mice (Figure 2, Table 2), which indicates that obese animals developed respiratory acidosis due to hypoventilation in the wake state. DIO mice had similar levels of V_E to lean mice across sleep/wake states, despite a nearly 60% increase in body weight and higher body temperature. Relationships between alveolar ventilation (V_A), CO_2 production (V_{CO2}) and alveolar CO_2 levels are defined by the alveolar ventilation equation $V_A \sim V_{CO2}/PaCO_2$. Increased body temperature in DIO mice in our study suggests an increase in metabolic rate and V_{CO2}, which is a common feature of DIO in humans and mice [31–33]. DIO mice did not increase V_E during sleep to compensate for increased metabolic rate. Thus, DIO mice have inadequately low ventilation during sleep and wakefulness, which explains hypoxemia.

There is a longstanding controversy as to whether OHS is due increased mechanical loads versus inadequate respiratory drive. Unlike humans, the mouse chest wall is infinitely compliant and there is no evidence that DIO has any effect on mouse respiratory mechanics [34], which suggests that DIO does not impose excessive mechanical loads on the respiratory pump muscles.

Table 3. Sleep architecture in lean and diet-induced obese C57BL/6J mice

Phenotype	Sleep efficiency (% of total time)	Sleep duration (min)			Sleep bouts			
					Number		Length (min)	
		Total	NREM	REM	NREM	REM	NREM	REM
Lean	54.0 ± 2.0	220 ± 11	208 ± 8	12.8 ± 4.0	71.0 ± 4.9	10.4 ± 4.5	3.0 ± 0.2	1.5 ± 0.2
Obese	38.0 ± 2.1	129 ± 13*	123 ± 13*	5.4 ± 11	55.0 ± 5.6*	5.0 ± 4.0	2.4 ± 0.3	1.4 ± 0.3

Values are presented as means ± *SEM*.
*$p < 0.05$.

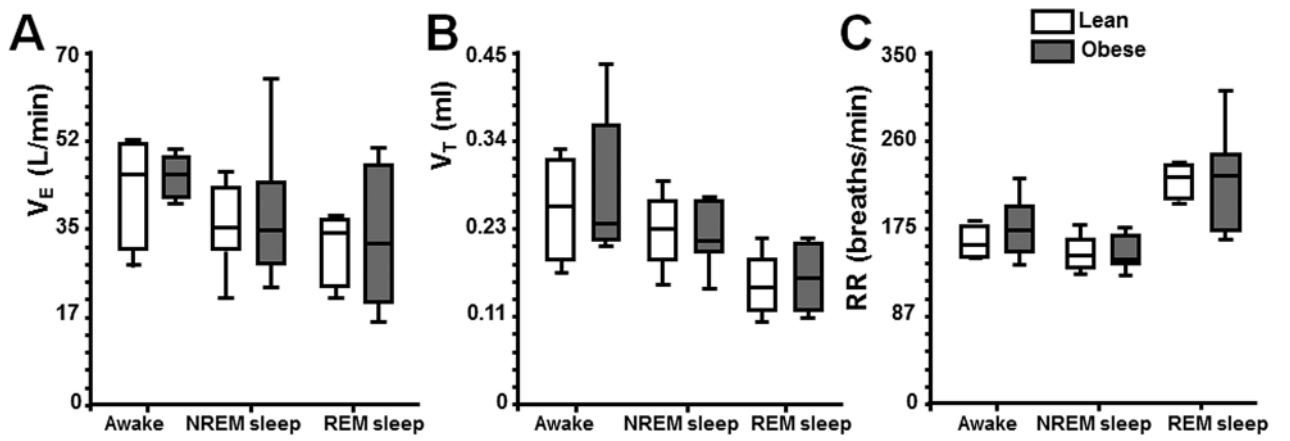

Figure 3. Characteristics of non-flow limited breathing in lean and diet-induced obese C57BL/6J mice. The box plot shows (A) V_E, (B) V_T, and (C) RR during non-flow limited breathing. The boxes denote the second and third quartile divided by the median. The whiskers denote the maximum and the minimum values.

Alternatively, OHS in humans has been associated with a defect in the ventilatory control during sleep [13, 35, 36]. Moreover, a degree of hypercapnia in patients with OHS was associated with high serum leptin levels and leptin resistance [18]. Leptin is a potent respiratory stimulant acting on medullary centers of hypercapnic sensitivity [14, 15, 37, 38]. Leptin-deficient *ob/ob* mice and leptin-resistant agouti yellow mice have a profound defect in the hypercapnic ventilatory response [15, 39]. We have previously measured the hypercapnic ventilatory response in DIO mice throughout sleep/wake states and did not find such a defect [34]. However, in our present study mice were 6 g heavier and had a sixfold higher leptin levels than in the previous report (Table 1). Leptin-deficient *ob/ob* mice develop OHS [29], which was more severe than hypoventilation in DIO mice in the current report. It is conceivable that both leptin deficiency and leptin resistance contribute to obesity hypoventilation. DIO mice may serve as a high throughput animal model to examine the role of leptin and leptin resistance in the pathogenesis of OHS.

IFL and compensatory responses

DIO significantly increased the prevalence of IFL breathing during NREM sleep, mimicking the effects of obesity on the upper airway function in humans. However, only 14.9% of breaths were flow limited in DIO mice and, therefore, IFL did not contribute significantly to hypoventilation during sleep. Previous data from our laboratory demonstrated that obesity increases pharyngeal collapsibility (critical pressure, P_{crit}) in sleeping humans [40–44] and in anesthetized mice [30, 45]. In obese patients, impaired upper airway function resulted in the high prevalence and severity of OSA compared to normal weight individuals [40–44], whereas weight loss significantly improved both pharyngeal collapsibility and OSA [42, 46, 47]. In contrast, DIO mice did not develop significant OSA. V_{Imax} was maintained during periods of IFL to levels observed in lean mice. The latter suggests that upper airway collapsibility remained sufficiently low to preserve ventilation and V_T during flow limited breathing and prevent oxygen desaturation (ODI). Despite elevations in passive P_{crit} in anesthetized obese mice [45], our previous study suggests that neural activation of upper airway muscles mitigated obstruction during sleep and prevented the development of frank OSA [45]. Of note, our current data also show a trend to an increase in ODI in DIO mice during REM sleep, when neuromuscular responses are diminished. This pattern of SDB was similar to that observed in children, which frequently show prolonged episodes of IFLs without apneic or hypopneic events [48, 49]. Thus, DIO in mice leads to IFL without upper airway obstruction of sufficient severity to cause periodic apneas and hypopneas.

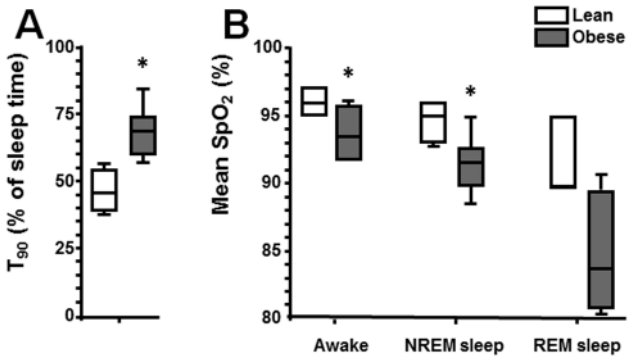

Figure 4. Characteristics of SpO_2 across sleep/wake states in lean and diet-induced obese C57BL/6J mice. The box plot shows (A) a percent of total sleep time spent with SpO_2 < 90% and (B) mean SpO_2 during wakefulness, NREM and REM sleep. The boxes denote the second and third quartile divided by the median. The whiskers denote the maximum and the minimum values. *$p < 0.05$ for the difference between lean and obese mice.

Limitations

First, our study was focused on SDB and was not designed to measure sleep patterns, which requires 24 hours recording. In fact, both DIO and genetic obesity due to the lack of leptin or leptin receptors leads to increased sleep time [50–53]. Our sleep recording was performed during the light predominantly restful phase in mice. However, other investigators demonstrated that high-fat diet disrupts circadian rhythm increasing activity and feeding during the light phase [54]. Our data indicating a decrease in NREM sleep time in DIO mice during the light phase may reflect a circadian rhythm disruption rather than curtailing of sleep time.

Second, we acknowledge that noninvasive measurements of respiratory effort can be useful only when mice are not moving (sleep and quiet wakefulness), whereas the signal becomes unreliable when animals shift their body positions [25].

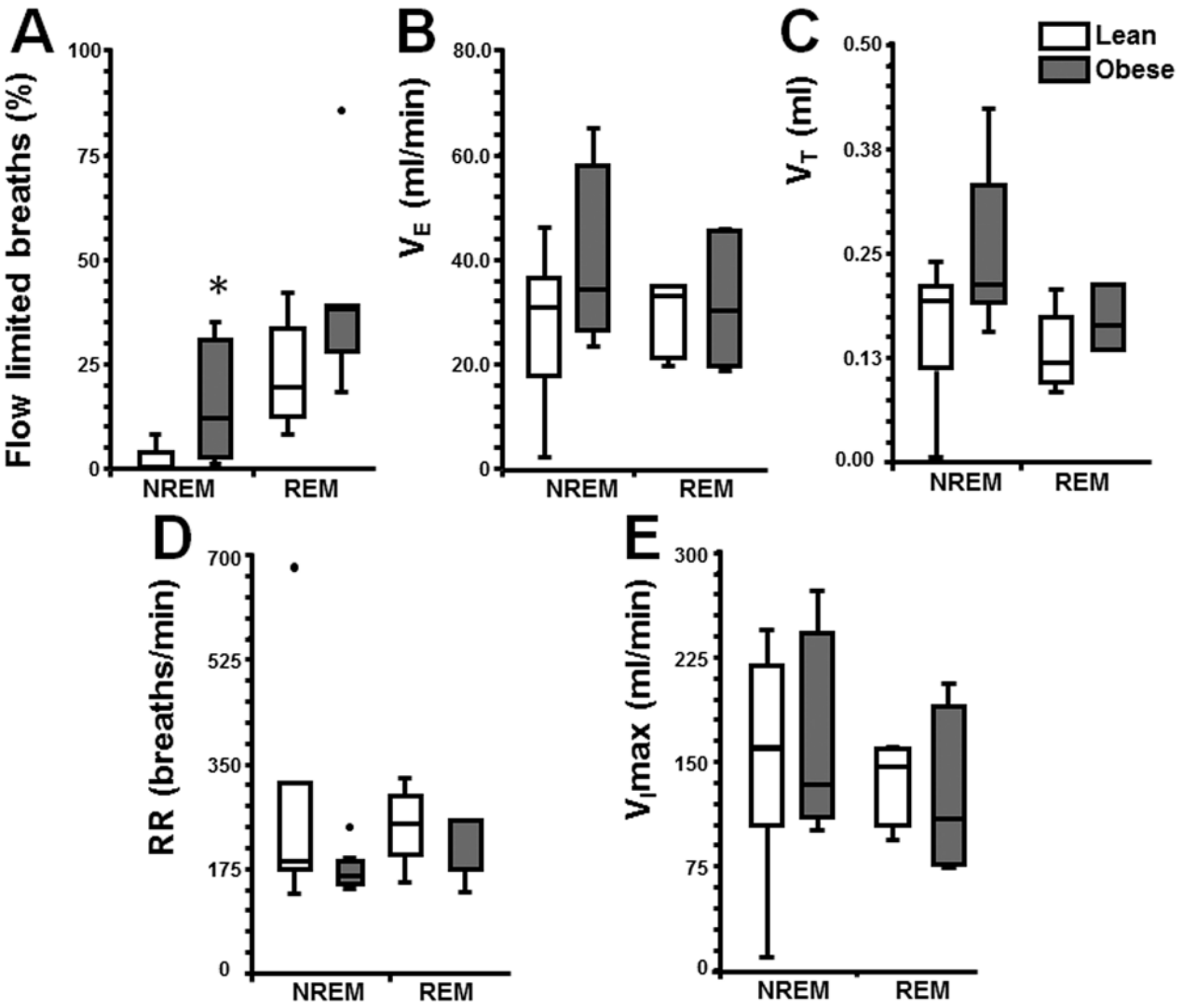

Figure 5. Characteristics of flow limited breathing in lean and diet-induced obese C57BL/6J mice. The box plot shows (A) a percent of breaths with inspiratory flow limitation; (B) V_E, (C) V_T, (D) RR during flow limited breathing, and (E) maximal inspiratory flow at the onset of flow limitation (V_I). The boxes denote the second and third quartile divided by the median. The whiskers denote the maximum and the minimum values. Outliers are defined as values exceeding the upper limit of the third quartile + 1.5× interquartile range and depicted as small solid circles. $^*p < 0.05$ for the difference between lean and obese mice.

Third, an unexpected finding was relatively high values for the time spent with SpO_2 < 90% (T90) in lean mice. This finding cannot be explained by poor signal quality or by IFL. Nevertheless, the oximeter yielded relative differences, which corresponded to the differences in blood gases.

Fourth, our data indicate that DIO mice hypoventilated during wakefulness, but we were unable to measure CO_2 levels during sleep. Nevertheless, inadequate levels of V_E in combination with low oxyhemoglobin saturation strongly suggest that hypoventilation worsened during sleep.

Fifth, our study describes a model of SDB in DIO mice, but the pathogenesis is not addressed. Future mechanistic work should address both anatomical and physiological causes of SDB in obesity, which may facilitate the development of novel treatment.

Conclusion

Severe DIO in mice lead to hypoventilation during wakefulness and sleep, whereas no significant upper airway obstruction was noted. Obesity hypoventilation in DIO mice is likely induced by inadequate drive to respiratory pump muscles. The DIO mouse may serve as a pre-clinical model of OHS in pharmacological studies.

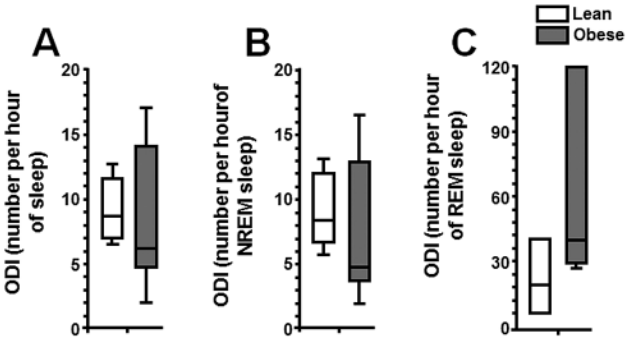

Figure 6. The ODI in lean and diet-induced obese C57BL/6J mice. The box plot shows ODI, which is a number of oxyhemoglobin desaturations ≥3% from baseline per hour for at least two breaths during (A) total sleep, (B) NREM sleep and (C) REM sleep. The boxes denote the second and third quartile divided by the median. The whiskers denote the maximum and the minimum values.

Supplementary material

Supplementary material is available at *SLEEP* online.

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Notes

Conflict of interest statement. None declared.

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