HIGHLIGHTED TOPIC | Upper Airway Control and Function: Implications for Sleep-Disordered Breathing

Leptin and the control of pharyngeal patency during sleep in severe obesity

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1Johns Hopkins Sleep Disorders Center, Division of Pulmonary & Critical Care Medicine, Johns Hopkins School of Medicine, Baltimore, Maryland; 2Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Kaohsiung, Taiwan; and 3Hospital Universitario (HU-USP), Sao Paulo, Brasil

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Shapiro SD, Chin C, Kirkness JP, McGinley BM, Patil SP, Polotsky VY, Biselli PJ, Smith PL, Schneider H, Schwartz AR. Leptin and the control of pharyngeal patency during sleep in severe obesity. J Appl Physiol 116: 1334–1341, 2014. First published February 20, 2014; doi:10.1152/japplphysiol.00958.2013.—Rationale: Obesity imposes mechanical loads on the upper airway, resulting in flow limitation and obstructive sleep apnea (OSA). In previous animal models, leptin has been considered to serve as a stimulant of ventilatory activity is reduced (33), increases 1.0 cmH₂O per 10 units increase in body mass index (BMI) in women and 1.7 cmH₂O in men (15). These increases may be due to fat deposits in the pharyngeal soft tissue, which increase the extraluminal tissue pressure (14) and augment mechanical loads on the upper airway (31). Additionally, central adiposity is associated with decreased lung volumes and diminished caudal traction, further increasing pharyngeal collapsibility (8, 38).

Obstructive sleep apnea (OSA) is characterized by repetitive upper airway obstruction during sleep, which results in recurrent oxyhemoglobin desaturations and arousals (30). Sleep apnea results from combined defects in upper airway anatomic and neuromuscular control during sleep (16). Obesity remains a predominant risk factor for OSA pathogenesis (23), although the mechanisms have not been well established.

Mechanical effects of obesity lead to elevations in the pharyngeal collapsibility (active PCRIT) during sleep in humans (15) and rodents (25, 28). Passive PCRIT, the nasal pressure at which the pharynx collapses when neuromuscular activity is reduced (33), increases 1.0 cmH₂O per 10 units increase in body mass index (BMI) in women and 1.7 cmH₂O in men (15). These increases may be due to fat deposits in the pharyngeal soft tissue, which increase the extraluminal tissue pressure (14) and augment mechanical loads on the upper airway (31). Additionally, central adiposity is associated with decreased lung volumes and diminished caudal traction, further increasing pharyngeal collapsibility (8, 38).

To compensate for pharyngeal mechanical loads in obesity, airflow obstruction can elicit neuromuscular responses that restore airway patency. Adipose tissue produces humoral factors including leptin, a powerful neurohumoral ventilatory stimulant in rodents. Of note, leptin-deficient ob/ob mice exhibit depressed ventilation and reduced responses to hypercapnia, even before the development of obesity (21, 26). Independent of changes in body weight, administration of exogenous leptin to these ob/ob mice increases pulmonary ventilation, restores normal responses to hypercapnia, and reverses defects in the neuromuscular control of the upper airway (24). Leptin’s actions on upper airway control and ventilatory drive may be centrally mediated by brain stem receptors in the nucleus tractus solitarius and hypoglossal motor nucleus (17). Inyushka et al. (12) demonstrated increased pulmonary ventilation, respiratory volume, and enhanced electromyography of the inspiratory muscles when leptin was directly injected into the ventrolateral solitary tract nucleus in the brain of rats. Nevertheless, the role of leptin in the regulation of upper airway neuromuscular control in sleeping humans is less well understood.

The major goal of this study is to examine the relationship between leptin and the control of upper airway patency during sleep in obesity. Previous studies have shown marked elevations in levels of circulating leptin (>100 ng/ml) in severe obesity (40) and marked variability in circulating leptin levels (4) independent of body weight. This variability may be related to differences in fat mass, fat distribution, and/or sleep apnea...
severity (4, 5, 13, 20, 40). We therefore hypothesized that in obese individuals, variations in individuals’ circulating leptin levels will be associated with differences in compensatory responses to upper airway obstruction during sleep. To address this hypothesis, we examined the relationship between circulating leptin and upper airway passive mechanical and active neuromuscular control in a severely obese bariatric cohort.

METHODS

Subjects

This study was a retrospective analysis of subjects previously recruited from the Johns Hopkins Sleep Disorders Center and the Johns Hopkins Bariatric Surgery Clinic for a variety of physiologic protocols. Because large variations in weight may influence both ventilatory parameters and circulating leptin levels, obese subjects were selected over a narrow range of BMI (40–50 kg/m²) to minimize potential confounds of differences in body weight on leptin concentrations (4, 40) and upper airway function (15, 32) during sleep. Subjects were excluded if they had a history of a concurrent sleep disorder (e.g., narcolepsy, restless leg syndrome, previous upper airway surgery, significant pulmonary disease or gas exchange abnormalities, or use of supplemental oxygen). Informed written consent was obtained from each participant, and the protocols were approved by the Johns Hopkins Institutional Review Board.

We characterized upper airway function in 61 bariatric candidates (50 women, 11 men), as reported in a previous publication (3). We selected the upper airway physiological data from 31 of those subjects (26 women, 5 men) whose BMI lay in the range targeted for our current study. Of these, active upper airway responses could not be determined during sleep because of repeated arousals (n = 2), inability to maintain stable breathing patterns at cycling threshold (n = 2), or excessive mouth breathing (n = 1), leaving 26 subjects (23 women, 3 men) in the final study group. Circulating leptin levels were assayed in this subgroup. The current report examines the relationship between measures of upper airway function during sleep and circulating leptin concentrations.

Study Procedures

Baseline polysomnography. A standard full-night baseline nocturnal polysomnography was performed in a sleep laboratory. Physiologic signals were digitized (Windaq, Dataq, Akron, OH, or Somnologica, Medcare, Buffalo, NY), including left and right electrooculogram, submental electromyogram, electroencephalogram, arterial oxygen saturation, nasal pressure, chest and abdominal plethysmography, and video monitoring for body position. Patients were instructed to sleep in the supine position, and efforts were made to maintain constant position between measurements of active and passive responses. All monitored parameters on this night were recorded directly on a computer software system.

Experimental Protocols

Experimental protocols were performed during stable non-rapid eye movement (NREM) sleep to assess compensatory responses to upper airway obstruction. Our experimental approach is detailed below and in Fig. 1 in a prior publication (3).

Passive condition—brief periods of upper airway obstruction. We first titrated nasal CPAP to a holding pressure that eliminated flow limitation and attenuated neuromuscular activity (36). Thereafter, nasal pressure was repeatedly acutely reduced from the holding pressure in 1- to 2-cmH₂O increments to lower levels each for five breaths; this stepwise reduction in nasal pressure occurred until complete upper airway closure (zero airflow) was induced, as previously described (17, 36, 37). If arousals occurred, the protocol was resumed after subjects returned to stable stage 2 NREM sleep for at least 3 min.

Active condition—sustained periods of upper airway obstruction. Nasal pressure was reduced stepwise from holding pressure by 1–2 cmH₂O for periods of at least 10 min during NREM sleep as previously described (17, 36, 37). Stepwise reductions in nasal pressure were associated with progressive decreases in maximal inspiratory airflow (VImax), as previously described.

Data Analyses

Assessment of compensatory responses. We utilized two methods for assessing compensatory upper airway responses. First, we measured baseline ventilatory characteristics during active and passive periods of airflow obstruction, as previously described (3). Specifically, we identified periods of non-flow-limited breathing during stable NREM sleep when nasal pressure was set to holding CPAP levels. We then determined ventilatory parameters in the active condition and measured ventilatory parameters during prolonged periods of partial airway obstruction (active condition). These parameters were determined at the cycling threshold pressure, the nasal pressure below which active ventilatory responses could no longer maintain stable ventilation during sleep. This threshold defined the limits of subjects’ ability to actively compensate for passive mechanical loads on the airway while stabilizing breathing patterns during sleep and represented the greatest degree of neuromuscular compensation during sleep (16). The degree of neuromuscular compensation was defined by the difference between VImax during the active and passive condition at the cycling threshold pressure (Fig. 1, see differences in compensatory responses, ΔVImax, between a subject with high compared with low leptin concentration). Specifically, passive and active flow-limited breaths were selected for analysis at the cycling threshold, as previously described (9).

Second, pressure-flow curves were derived for the passive and active conditions from previously established methods (22). In each condition, pressure-flow measurements at the cycling threshold pressure and standardized values for upstream resistance (22) were utilized to derive passive and active critical pressure (Pcrit). The compensatory response was given by the difference between the active and passive Pcrit for each subject (ΔPcrit).

In addition, we examined the association between leptin concentrations and ventilatory drive, as reflected by inspiratory swings in esophageal pressure. We compared inspiratory swings in esophageal pressure for each subject during the active condition, when drive was...
maximal, to the corresponding passive state, when neuromuscular activity was minimal. The difference in esophageal pressure swings between these two conditions was taken as a measure of ventilatory compensation to airflow obstruction.

Statistical analysis. Our analyses focused on testing the associations between measures of passive and active upper airway control and circulating leptin concentration, as well as other measures of adiposity and sleep apnea severity (STATA 11, College Station, TX). The primary outcome variables were passive PCRIT and compensatory neuromuscular responses, as represented by the differences in inspired minute ventilation ($\Delta V_I$), maximum inspiratory airflow ($\Delta V_{I\text{max}}$), and pharyngeal collapsibility ($\Delta P_{\text{CRIT}}$) between active and passive conditions. Predictors of upper airway function included serum leptin concentration, BMI, waist-to-hip ratio (WHR), sagittal girth (girth), and neck circumference (neck). The strength of the associations between predictors and outcome variables was analyzed with categorical and linear regression models. Multivariable regression models were used to assess the associations between compensatory neuromuscular responses and leptin concentration while adjusting for measures of adiposity (BMI, WHR, girth, neck). Finally, our sample contained relatively few men ($n = 3$), which limited our ability to investigate sex differences in the associations between compensatory responses and leptin concentration. We therefore provide data from the men but exclude them from our analysis, because the sample size limited our ability to discern sex-related differences in compensatory responses. Values were expressed as means ± SD unless otherwise stated, and $P < 0.05$ was considered significant.

RESULTS

Subject Characteristics

The anthropometric data, baseline sleep characteristics, and serum leptin concentration of the 26 subjects (23 women, 3 men) who comprised our study group are displayed in Table 1. By design, subjects were severely obese with an average BMI of 46 kg/m² for both sexes. They had moderate to severe sleep apnea ($\text{AHI} = 25 ± 26/69 ± 33$; women/men) and elevated leptin concentrations, with women exhibiting a roughly two-fold elevation over men. Spirometry demonstrated that subjects’ vital capacity was relatively well preserved and none had evidence of an obstructive ventilatory defect (i.e., the FEV1/FVC ratio was $>70\%$). Arterial blood gases did not demonstrate evidence of alveolar hypoventilation in any of the women (i.e., $\text{PaCO}_2$ was $<45$ mmHg), although one of the men had evidence of a mild well-compensated respiratory acidosis with a $\text{PaCO}_2$ of 49 mmHg and a pH of 7.38. Thus spirometry and arterial blood gas results did not suggest the presence of underlying structural lung disease or the obesity-hypoventilation syndrome in the women, who comprised the study group of interest.

Leptin, Adiposity, and Sleep-Disordered Breathing

As expected, increasing levels of obesity were associated with increased concentrations of leptin. In women, serum leptin was positively associated with BMI ($r^2 = 0.24$, $P < 0.02$, $\beta = 5.3 ± 2.0$ ng/ml per kg/m²). However, leptin concentration was not associated with measures of body composition, including sagittal girth ($r^2 = 0.09$, $P < 0.19$, $\beta = 2.5 ± 2.1$ ng/ml per cm), neck ($r^2 = 0.10$, $P < 0.16$, $\beta = -3.6 ± 2.4$ ng/ml per cm), and WHR ($r^2 = 0.07$, $P < 0.24$, $\beta = -94 ± 77$ (ng/ml)). In multiple regression models, the association between serum leptin concentration and BMI remained significant after adjusting for WHR ($P < 0.009$, $\beta = 5.7 ± 2.0$ ng/ml per kg/m²), neck ($P < 0.034$, $\beta = 4.8 ± 2.4$ ng/ml per kg/m²), and girth ($P < 0.028$, $\beta = 5.0 ± 2.1$ ng/ml per kg/m²).

In women, leptin concentration was not associated with sleep-disordered breathing severity as reflected by the apnea-hypopnea index (AHI) in non-REM ($r^2 = 0.31$, REM ($r^2 < 0.001$, $P < 0.94$), or in non-REM and REM sleep combined ($r^2 = 0.03$, $P < 0.56$). Additionally, no associations were observed between leptin concentration and either baseline oxygen saturation or average low oxygen saturation during sleep-disordered breathing episodes in either non-REM or REM sleep.

Upper Airway Characteristics and Sleep-Disordered Breathing

Sleep apnea was absent in only one patient, whose passive $P_{\text{CRIT}}$ was below $-4$ cmH₂O, a previously described threshold for the development of sleep apnea (7, 22). In contrast, sleep-disordered breathing was present, but AHI varied widely in severity in patients whose passive $P_{\text{CRIT}}$ exceeded this threshold ($n = 25$), suggesting varying degrees of active neuromuscular responses. Variability in these responses can account for...
Table 1. Subject demographics

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Women (n = 22)</th>
<th>Men (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>41.2 ± 11.7 (33.9–51)</td>
<td>43.1 ± 3.7 (38.9–45.7)</td>
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<tr>
<td>Anthropometrics</td>
<td></td>
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</tr>
<tr>
<td>BMI, kg/m²</td>
<td>45.9 ± 3.0 (44.0–48.4)</td>
<td>46.1 ± 2.6 (44.2–49.1)</td>
</tr>
<tr>
<td>Neck, cm</td>
<td>39.4 ± 2.8 (38.0–40.7)</td>
<td>46.4 ± 1.8 (44.5–48.1)</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>128.6 ± 24.2 (118.4–133.3)</td>
<td>139.4 ± 11.1 (126.8–147.7)</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.9 ± 0.1 (0.9–0.9)</td>
<td>1.0 ± 0.0 (1.0–1.0)</td>
</tr>
<tr>
<td>Girth, cm</td>
<td>34.0 ± 20.1 (28.0–32.0)</td>
<td>33.3 ± 4.0 (29.0–37.0)</td>
</tr>
<tr>
<td>Sleep architecture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TST, min</td>
<td>394.4 ± 65.5 (367.5–442.4)</td>
<td>425.3 ± 100.0 (358.0–540.0)</td>
</tr>
<tr>
<td>Sleep efficiency, %</td>
<td>85.2 ± 12.7 (82.0–93.0)</td>
<td>94.8 ± 2.4 (92.0–96.5)</td>
</tr>
<tr>
<td>Stage N1, %TST</td>
<td>15.0 ± 11.0 (9.5–16.7)</td>
<td>21.6 ± 13.8 (5.7–30.6)</td>
</tr>
<tr>
<td>Stage N2, %TST</td>
<td>56.8 ± 7.6 (51.1–62.0)</td>
<td>55.1 ± 10.0 (46.1–65.8)</td>
</tr>
<tr>
<td>Stage N3, %TST</td>
<td>11.5 ± 10.3 (1.3–19.2)</td>
<td>3.3 ± 5.7 (0.9–9.8)</td>
</tr>
<tr>
<td>REM, %TST</td>
<td>16.7 ± 8.3 (11.2–23.7)</td>
<td>20.0 ± 4.9 (15.9–25.4)</td>
</tr>
</tbody>
</table>

Sleep-Disordered Breathing

- Non-REM
  - AHI events/h: 22 ± 28 (5–23) vs. 65.4 ± 39.4 (30.7–108.2)
  - Baseline SaO2: 96.0 ± 1.9 (94.7–97.5) vs. 95.1 ± 0.9 (94.3–96.0)
  - Average low SaO2: 91.7 ± 2.4 (89.4–93.6) vs. 87.4 ± 3.9 (83.4–91.1)

- REM
  - AHI events/h: 32 ± 23 (10–47)* vs. 80 ± 12 (68–92)
  - Baseline SaO2: 95.7 ± 1.9 (95.0–97.4)* vs. 93.8 ± 1.8 (91.8–95.2)
  - Average low SaO2: 90.0 ± 2.9 (88.6–91.4)* vs. 82.5 ± 5.3 (79.4–88.6)

- Total
  - AHI events/h: 24.8 ± 26.2 (6.4–31.6) vs. 69 ± 33 (43–106)
  - Baseline SaO2: 95.9 ± 1.9 (95.0–97.3) vs. 94.7 ± 0.8 (94.3–95.6)
  - Average low SaO2: 90.9 ± 2.2 (89.7–92.8) vs. 86.2 ± 3.5 (83.0–89.9)

Arterial Blood Gases (19 women/2 men)

- $P_{O_2}$: 86.6 ± 13.2 (77.5–94.5) vs. 73.5 ± 4.95 (70.0–77.0)
- $P_{CO_2}$: 38.9 ± 5.12 (37.0–42.5) vs. 45.5 ± 4.95 (42.0–49.0)
- pH: 7.42 ± 0.04 (7.40–7.44) vs. 7.40 ± 0.02 (7.38–7.41)

Pulmonary Function Tests (21 women, 3 men)

- FVC, liters: 3.42 ± 0.71 (2.88–3.92) vs. 5.09 ± 0.12 (5.05–15.6)
- FVC predicted, %: 96.4 ± 16.4 (88.0–112.9) vs. 104 ± 3.90 (102.9–106.3)
- FEV₁, liters: 2.77 ± 0.55 (2.40–3.09) vs. 3.90 ± 0.04 (3.89–3.93)
- FEV₁ predicted, %: 93.5 ± 14.6 (82.0–101.8) vs. 96.2 ± 1.72 (95.3–97.0)
- FEV₁/FVC, %: 81.4 ± 4.70 (77.8–84.6) vs. 86.6 ± 13.2 (75.4–77.8)
- Serum Leptin, ng/ml: 95.2 ± 32.6 (74.2–114.2) vs. 43.4 ± 10.3 (31.6–50.2)
- Passive $P_{CRRT}$, cmH₂O: 0.2 ± 3.3 (–2.5–1.7) vs. –1.1 ± 2.2 (–3.2–1.2)
- Active $P_{CRRT}$, cmH₂O: –2.9 ± 0.9 (–5.5–0.7) vs. –1.9 ± 1.1 (–3.1–3.1)

All values are presented as mean ± SD (25–75th percentile). AHI, apnea-hypopnea index; BMI, body mass index; SaO₂, oxygen saturation; TST, total sleep time. *Three women had no rapid eye movement (REM) sleep.

Altersations in $V_{Imax}$ under active conditions, which was a strong predictor of AHI (non-REM and REM combined). Specifically, subjects with low compared with high $V_{Imax}$ at atmospheric pressure during sleep (median, 158 ml/s; low $V_{Imax}$, 38 ± 51 ml/s; high $V_{Imax}$, 278 ± 115 ml/s) had significantly higher AHI (53 ± 33 vs. 14 ± 8 episodes/h, $P < 0.001$).

**Leptin, Ventilation, and Compensatory Responses to Upper Airway Obstruction**

At holding pressure, baseline ventilatory characteristics were not associated with leptin concentration in women, including minute ventilation ($V_i$) ($r^2 = 0.004, P < 0.76$) and $V_{Imax}$ at the cycling threshold ($r^2 < 0.001, P < 0.99$). Similarly, passive $P_{CRRT}$ was not associated with leptin concentration ($r^2 < 0.002, P < 0.80$), suggesting that leptin does not determine upper airway mechanical loads in severe obesity.

Compensatory responses to airflow obstruction were measured as the differences in ventilatory parameters between active and passive conditions, as shown in Fig. 1. In this figure, an augmented $Δ V_{Imax}$ response is illustrated in a subject with a high leptin (Fig. 1, top) compared with a subject with a low leptin (Fig. 1, bottom) concentration (156 vs. 35 ng/ml, respectively). In each subject, an abrupt drop in nasal pressure was associated with the onset of inspiratory flow limitation in the passive condition (Fig. 1, left). In contrast, maintaining nasal pressure near the cycling threshold (Fig. 1, right) resulted in an increase in $V_{Imax}$ or little change in $V_{Imax}$ in the subject with a high compared with low leptin level (Fig. 1, right vs. bottom, respectively). The comparatively greater $Δ V_{Imax}$ response in the subject with high compared with low leptin concentration reflects a greater compensatory upper airway response to airflow obstruction.

Examining compensatory responses for the group as a whole, we found that $Δ V_{Imax}$ was highly associated with serum leptin concentrations (Table 2) but not with indices of obesity (BMI) or fat distribution (WHR, girth, neck). Compensatory responses in $V_{Imax}$, $V_i$, and $P_{CRRT}$ were each associated with increasing leptin concentrations in the women (Fig. 2). Even after removing the two women with the highest leptin levels from these analyses as potential outliers, compensatory responses in $Δ V_{Imax}$ and $Δ V_i$ remained positively associated.
with circulating leptin levels \( (P < 0.03 \text{ and } <0.01, \text{ respectively}) \), although the \( \Delta \text{PCRIT} \) association lost significance \( (P = 0.27) \). Compared with women, we found that men demonstrated low leptin concentrations and airflow responses to upper airway obstruction \( (\Delta V_{\text{Imax}} \text{ and } \Delta \text{PCRIT}) \). Despite reductions in upper airway responses \( (\Delta V_{\text{Imax}}) \), the men’s ventilatory responses \( (\Delta V_{I}) \) were elevated, consistent with alterations in respiratory pattern that compensated for upper airway obstruction \( \text{e.g.}, \text{increased inspiratory duty cycle} \). Increases in leptin were also associated with more negative \( \Delta \text{PCRIT} \), indicating augmented compensatory responses that decreased active \( P_{\text{CRIT}} \) below passive levels.

Circulating leptin concentrations were also associated with increases in ventilatory drive, as represented by the different esophageal pressure swings between passive and active conditions. A trend toward a significant linear relationship was

### Table 2. Regression models for compensatory responses in women

<table>
<thead>
<tr>
<th>( \Delta V_{I}, \text{l/min} )</th>
<th>( \beta )</th>
<th>95% CI</th>
<th>( r^2 )</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin, ng/ml</td>
<td>0.017</td>
<td>0.008–0.027</td>
<td>0.400</td>
<td>0.001</td>
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<tr>
<td>BMI, kg/m²</td>
<td>0.011</td>
<td>-0.121–0.143</td>
<td>0.001</td>
<td>0.864</td>
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<tr>
<td>Girth, cm</td>
<td>0.090</td>
<td>-0.022–0.204</td>
<td>0.117</td>
<td>0.110</td>
</tr>
<tr>
<td>Neck, cm</td>
<td>0.018</td>
<td>-0.125–0.161</td>
<td>0.003</td>
<td>0.795</td>
</tr>
<tr>
<td>WHR</td>
<td>-3.790</td>
<td>-7.954–0.373</td>
<td>0.146</td>
<td>0.072</td>
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</table>

<table>
<thead>
<tr>
<th>( \Delta V_{\text{Imax}}, \text{ml/s} )</th>
<th>( \beta )</th>
<th>95% CI</th>
<th>( r^2 )</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin, ng/ml</td>
<td>0.83</td>
<td>0.41–1.26</td>
<td>0.444</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>1.98</td>
<td>-4.09–8.06</td>
<td>0.021</td>
<td>0.504</td>
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<tr>
<td>Girth, cm</td>
<td>3.92</td>
<td>-1.39–9.24</td>
<td>0.101</td>
<td>0.139</td>
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<tr>
<td>Neck, cm</td>
<td>-1.84</td>
<td>-8.43–4.76</td>
<td>0.016</td>
<td>0.569</td>
</tr>
<tr>
<td>WHR</td>
<td>-16.6</td>
<td>-225–192</td>
<td>0.001</td>
<td>0.870</td>
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</tbody>
</table>

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<thead>
<tr>
<th>( \Delta \text{PCRIT}, \text{cmH}_2\text{O} )</th>
<th>( \beta )</th>
<th>95% CI</th>
<th>( r^2 )</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin, ng/ml</td>
<td>-0.02</td>
<td>-0.1–0.0</td>
<td>0.190</td>
<td>0.036</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>-0.07</td>
<td>-0.3–0.2</td>
<td>0.010</td>
<td>0.592</td>
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<tr>
<td>Girth, cm</td>
<td>-0.05</td>
<td>-0.3–0.2</td>
<td>0.001</td>
<td>0.681</td>
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<tr>
<td>Neck, cm</td>
<td>0.17</td>
<td>-0.1–0.5</td>
<td>0.070</td>
<td>0.224</td>
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<tr>
<td>WHR</td>
<td>-4.3</td>
<td>-13.5–4.8</td>
<td>0.040</td>
<td>0.335</td>
</tr>
</tbody>
</table>

\( \Delta V_{\text{Imax}}, \text{maximum inspiratory airflow}; \Delta V_{I}, \text{minute ventilation}; \Delta \text{PCRIT}, \text{pharyngeal critical pressure}; \text{Girth, sagittal girth}; \text{Neck, neck circumference}; \text{WHR, waist-to-hip ratio}. \)

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![Fig. 2](https://jap.physiology.org/)

**Fig. 2.** Compensatory responses \( \Delta V_{\text{Imax}}, \text{minute ventilation (}\Delta V_{I}\text{)}, \text{and upper airway critical pressure (}\Delta \text{PCRIT}) \) were significantly associated with serum leptin concentration in 23 women (○). Statistical results represent data from women only. Data in 3 men (○) are also shown.
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found between esophageal pressure differences and leptin levels for the entire group (men and women combined, n = 19), such that progressive increases in these pressure swings were associated with increased leptin concentrations (β coefficient: 0.09 ± 0.5 cmH2O per ng/ml, P = 0.06). This relationship was largely unaltered after adjusting for concomitant increases in upper airway patency (ΔVmax) (β coefficient: 0.10 ± 0.5 cmH2O per ng/ml, P = 0.07). Restricting our analysis to the women alone (n = 17), this association did not achieve statistical significance, although the magnitude of the association was similar to that of the entire group (β coefficient: 0.09 ± 0.8 cmH2O per ng/ml, P = 0.16). These findings suggest that leptin levels might predict active responses in ventilatory drive during sustained periods of airway obstruction, although associations were less pronounced than those between compensatory upper airway neuromuscular responses and leptin levels.

In multivariable regression models, leptin concentration remained significantly associated with these compensatory responses after adjusting for specific measures of obesity and fat distribution in the obese women. In these multivariable regression models, BMI, WHR, girth, and neck were not associated with compensatory neuromuscular responses nor did they significantly alter point estimates of the β coefficient for leptin responses in Vmax (7.90–9.80 ml/s per 10 ng/ml) and V1 (0.15–0.22 l/min per 10 ng/ml) and PCRIT (–0.2–0.29 cmH2O per 10 ng/ml). These findings suggest that leptin increases compensatory neuromuscular responses to upper airway obstruction during sleep, independent of measures of obesity and regional fat distribution.

DISCUSSION

In the current study, we demonstrated a significant association between upper airway neuromuscular responses and circulating leptin concentrations in obese women during sleep. These responses were independent of other measures of adiposity including BMI, WHR, neck circumference, and sagittal girth. Furthermore, leptin was not associated with baseline measures of sleep-disordered breathing severity, passive PCRIT, or ventilation. These results suggest that leptin is either a marker or mediator of upper airway compensatory neuromuscular responses and that the neurohormonal actions of leptin can minimize upper airway collapse and restore ventilation during sleep.

Obesity imposes mechanical load on the pharynx that increases its collapsibility, causing airflow obstruction during sleep (34). Increases in passive PCRIT are associated with increased sleep apnea severity, independent of leptin concentration. In our obese subjects, passive PCRIT in women was atmospheric, implying that the airway would occlude completely when neuromuscular activity wanes during sleep. Airflow obstruction, however, elicited variable increases in Vmax and minute ventilation that mitigated the effects of elevated mechanical loads on airflow obstruction. We found that these active responses were directly related to circulating leptin concentration rather than baseline ventilatory parameters at holding pressure or measures of adiposity and fat distribution. These findings suggest that leptin may compensate for passive mechanical loads on the upper airway by augmenting active responses to obstruction, thereby minimizing the potential severity of the disease in obese individuals. Nevertheless, these compensatory responses may not be sufficient to prevent upper airflow obstruction if mechanical loads on the upper airway (passive PCRIT) are markedly elevated.

Obstructive sleep apnea and obesity are both associated with marked increases in circulating leptin concentration (1, 4), raising the possibility that OSA may confound the relationship between leptin and active neuromuscular responses. In addition, sleep apnea (13) and nocturnal intermittent hypoxia (29) are thought to induce a leptin-resistant state (19), which if present would attenuate our association between leptin and compensatory neuromuscular responses. Instead, we found no relationship between serum leptin concentration and nocturnal hypoxemia (see RESULTS). Rather, increases in leptin concentration were associated with increases in compensatory responses, which could potentially ameliorate OSA (22). These findings are consistent with the notion that leptin contributes to active upper airway responses during sleep and that any further increases in leptin from sleep apnea would serve to mitigate the severity of airflow obstruction during sleep (35).

In severe obesity, circulating leptin concentrations can vary considerably among similarly obese individuals (4, 40). This variability has been attributed to differences in regional adiposity, because elevations in circulating leptin levels have been associated with a predominance of subcutaneous compared with visceral fat (6, 18). Women, who generally have more subcutaneous fat than men (20), exhibit significantly higher circulating leptin levels (27). Women also have far greater active neuromuscular responses to obstruction (3), thereby potentially contributing to the decreased sleep apnea prevalence and severity in women compared with men (15). These findings suggest that leptin may mediate reductions in OSA susceptibility in women by increasing neuromuscular responses to upper airflow obstruction.

Several factors may limit the interpretation of our findings. First, our sample size was small and restricted to a severely obese population, limiting our ability to generalize our findings to less obese cohorts. The small sample size may have also limited our ability to discern a significant relationship between ventilatory drive and serum leptin as has been reported in murine models (11, 12, 21, 39). Nonetheless, our findings suggest that leptin’s effect on ventilatory responses to airflow obstruction was less pronounced than that on compensatory upper airway neuromuscular responses during sleep. Second, the lack of men in our study prevented us from characterizing their relationship between leptin and neuromuscular compensatory responses and from conducting a direct comparison of sex-related differences. Post hoc analysis showed that statistical significance would still be observed in both primary outcome parameters (ΔV1 and ΔVmax and ΔPCRIT) were men included in the overall analysis. Additional men will need to be recruited from the bariatric surgery cohort for future studies. Third, we did not control for the menstrual cycle and menopausal status in women. It is possible that hormonal differences and the inclusion of postmenopausal women may have influenced our serum leptin levels and associated compensatory responses. Likewise, we acknowledge the daily rhythmic variations in circulating leptin levels. Current literature suggests that leptin levels are influenced by the interaction of circadian rhythms, feeding patterns, and sleep time, with a nadir in the

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morning and a peak at night. To control for these fluctuations, patient’s blood was drawn in the mornings immediately after their baseline study. This allowed us to standardize subjects’ leptin measurements to a morning condition after an 8-h observed fasting period. Fourth, circulating leptin concentrations may not accurately reflect central nervous system levels. Active responses are thought to be centrally mediated; if leptin modulates these responses, it would need to be transported across the blood-brain barrier (2). Fifth, although we found that compensatory neuromuscular responses were highly associated with circulating leptin concentration rather than measures of regional fat distribution, it is certainly possible that other adipokines or measures of regional adiposity could also predict these responses. Sixth, we recognize that sleep apnea and its treatment may confound the relationship between compensatory neuromuscular responses and leptin concentration. Nevertheless, our female subjects had relatively mild sleep apnea, and their disease severity was not associated with circulating leptin concentration. Seventh, we acknowledge the positional effects of upper airway collapse. Patients were instructed to sleep supine if possible, and great efforts were made to maintain consistent position between the different sleep nights, as well as during the active and passive measurements. Given the nature of our study design, each subject served as his own baseline control, allowing us to control for positional and mechanical variability among the subjects. The observed compensatory responses were thus purely neurally derived. Eighth, it is also possible that leptin levels were associated with increases in ventilatory as well as upper airway compensatory responses. We sought to quantify differences in ventilatory responses between active and passive conditions from measurements of esophageal pressure excursions within the first tenth of a second after dropping the nasal pressure to induce airway occlusion ($P_{0.1}$). These measurements, however, were difficult to standardize due to variability in the degree of airway obstruction ($n = 9$), signal artifacts ($n = 2$), and lung deflation transients immediately after abrupt decreases in nasal pressure. Measurements were not possible in other subjects, who were unable to tolerate the esophageal catheter ($n = 5$). Nonetheless, we did not discern any significant relationship between compensatory $P_{0.1}$ responses and circulating leptin concentrations in our remaining subject sample ($n = 10$). Finally, we acknowledge that observed cross-sectional associations between neuromuscular responses and leptin concentration cannot establish causality. Future studies are needed to determine if leptin has a direct effect on compensatory responses to upper airway obstruction during sleep, as suggested by recent findings in a mouse model (24).

In conclusion, our study demonstrated that increased circulating leptin levels were associated with heightened ventilatory responses to upper airway obstruction. Leptin may augment compensatory neural mechanisms in response to upper airway obstruction during NREM sleep, thereby potentially minimizing upper airway collapse and mitigating potential OSA severity. Variability in leptin concentration among similarly obese individuals may be related to alterations in regional fat and can account for differences in OSA susceptibility between obese women and men. Further research is required to determine leptin’s influence on ventilation and upper airway neuromuscular control over a broad range of subjects and potential treatment effects in human diseases.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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