Effects of leptin and obesity on the upper airway function

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Polotsky M, Elsayed-Ahmed AS, Pichard L, Harris CC, Smith PL, Schneider H, Kirkness JP, Polotsky V, Schwartz AR. Effects of leptin and obesity on the upper airway function. J Appl Physiol 112: 1637–1643, 2012.—Obesity is associated with alterations in upper airway collapsibility during sleep. Obese, leptin-deficient mice demonstrate blunted ventilatory control, leading us to hypothesize that (1) obesity and leptin deficiency would predispose to worsening neuromechanical upper airway function and that (2) leptin replacement would acutely reverse neuromuscular defects in the absence of weight loss. In age-matched, anesthetized, spontaneously breathing C57BL/6J (BL6) and ob−/ob− mice, we characterized upper airway pressure-flow dynamics during ramp decreases in nasal pressure (PN) to determine the passive expiratory critical pressure (PCRT) and active responses to reductions in PN, including the percentage of ramps showing inspiratory flow limitation (IFL; frequency), the PN threshold at which IFL developed, maximum inspiratory airflow (VImax), and genioglossus electromyographic (EMGGG) activity. Elevations in body weight were associated with progressive elevations in PCRT (0.1 ± 0.02 cmH2O/g), independent of mouse strain. PCRT was also elevated in ob−/ob− compared with BL6 mice (1.6 ± 0.1 cmH2O/g), independent of weight. Both obesity and leptin deficiency were associated with significantly higher IFL frequency and PN threshold and lower VImax. Very obese ob−/ob− mice treated with leptin compared with nontreated mice showed a decrease in IFL frequency (from 63.5 ± 2.9 to 30.0 ± 8.6%) and PN threshold (from −0.8 ± 1.1 to −5.6 ± 0.8 cmH2O) and increase in VImax (from 354.1 ± 25.3 to 659.0 ± 71.8 μL/s). Nevertheless, passive PCRT in leptin-treated mice did not differ significantly from that seen in nontreated ob−/ob− mice. The findings suggest that weight and leptin deficiency produced defects in upper airway neuromechanical control and that leptin reversed defects in active neuromuscular responses acutely without reducing mechanical loads.

Obstructive sleep apnea; neuromuscular control; leptin; ob−/ob−

Effect of weight management protocol on the upper airway function of C57BL/6J and ob−/ob− mice

Male C57BL/6J (BL6) and ob−/ob− leptin-deficient mice were obtained from Jackson Laboratory (Bar Harbor, ME) and housed in a micro-isolation facility. Temperature and humidity were continuously regulated at 20–22°C and 40–60% relative humidity, respectively. Both groups of mice were utilized at 9 wk of age. Dietary intake was managed to achieve the target weight goals of lean (~24 g) and obese (~30 g) in BL6 mice and lean (~24 g), obese (~30 g), and very obese (~40 g) in ob−/ob− mice (see Weight management protocol below). Water was available ad libitum throughout the study for all groups. All study protocols were approved by the Johns Hopkins Animal Care and Use Committee (JHACUC) and all animal experiments were conducted in accordance with the JHACUC guidelines. Data from BL6 mice also served as controls for a concurrent study on mouse upper airway control (29).

Experimental Procedures

Weight management protocol. BL6 and ob−/ob− mice arrived from The Jackson Laboratory at 7 wk of age with an average weight of 17 and 35 g, respectively. Mice on normal chow (18% protein extruded rodent diet, Harlan Laboratories, Madison, WI) naturally gained weight over time at a predictable rate (11). To augment weight, a

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high-fat diet (35% lard diet, Harlan Laboratories, Madison, WI) was administered and resulted in the development of an "obese" group of BL6 mice. The ob+/ob+ mice were placed in separate cages, in which they were weighed daily and caloric intake was regulated accordingly to develop "lean," "obese," and "very obese" groups. The final ages and weights for each target group are represented in Table 1.

Anesthesia protocol. Isoflurane was used to induct and maintain anesthesia, as previously described (29). Atropine was injected (0.001 mg ip) to minimize airway secretions, rectal temperature was monitored, and body temperature was maintained at 36.5–37.5°C with a variable temperature heating pad. At the experiment’s completion, the animals were euthanized by an overdose of pentobarbital sodium (60 mg ip).

Intact murine upper airway surgery. The trachea was cannulated with a tapered cannula through a midline incision and secured, as previously described (29). Two Teflon-coated fine wires were tunneled subcutaneously and sutured to the ventral surface of the genioglossus muscle bilaterally. Surface electrodes were utilized to monitor activity from the geniohyoid/genioglossus group (rather than intrinsic tongue muscles) and to minimize potential mechanical artifact from hook electrodes, which would have had to be inserted in the tongue body itself. The mouth was sewn shut and the lips were sealed with acrylic adhesive.

Leptin treatment. In a group of 8 very obese ob+/ob+ mice, a 100-μl Alzet (1 μl/h) osmotic minipump (Mt. View, CA) filled with mouse recombinant leptin (R&D) was inserted under isoflurane anesthesia through a small midline incision, just caudal to the shoulder blades. Over a period of 3 days, the pump released leptin at a rate of 30 μg/day, leading to a predictable increase in leptin concentration, as previously described (22).

Experimental Setup

The mouse was placed into a head-out plethysmograph in the prone position, as previously described (29). The mouth was sealed and a low-density nasal cannula was placed over the snout and secured with acrylic adhesive. The cannula was connected to a blow-by breathing circuit through which fresh oxygen and isoflurane were administered. The nasal pressure (Pn) and tracheal pressure (Ptrach) were monitored with differential pressure transducers referenced to atmospheric pressure. To regulate the Pn, the downstream end of the blow-by circuit was connected in series to a rotometer and vacuum source. Inspiratory airflow (Vi) was measured through a fixed resistance, calibrated laminar flow pneumotachometer attached to a hole in the plethysmograph and monitored with a differential pressure transducer. Pn, Ptrach, and Vi were amplified and digitized for real-time display, storage, and data analysis. During the experimental protocol (see below), isoflurane anesthesia was titrated between 1 and 2% to control the respiratory rate between 40 and 90 breaths/min. The patency of the tracheostomy tube was maintained throughout the experiments by flushing the tracheal cannula with air for ~1 s immediately prior to each negative pressure ramp (see below) and by aspirating secretions as needed.

Electromyography. The genioglossus electromyographic activity (EMGgg) signal was amplified, band-pass-filtered from 30 to 1,000 Hz, and digitized at a sampling rate of 1,000 Hz. The signal was rectified, and a 55-ms time constant was applied to compute the moving average.

Experimental Protocol

Passive and active upper airway function was assessed, as previously described (29). In brief, passive upper airway function was assessed during expiration (33), when EMGgg fell to tonic levels. Pn was manipulated to vary the state of upper airway patency. Pn was lowered in ramplike fashion from approximately +10 cmH2O to approximately −30 cmH2O (see Fig. 1 in Ref. 29). The passive Pcrit was defined by the end-expiratory Ptrach plateau, when Ptrach and Pn signals diverged (the pharynx occluded) with further decreases in Pn (15, 33, 34). We previously demonstrated that oropharyngeal pressure also dissociated from the nasal pressure during this maneuver, indicating that the flow limiting site was rostral to the palatal rim during expiration (29). Approximately 10 Pn ramps were performed to characterize passive Pcrit in each mouse.

Active upper airway function was assessed during inspiration, when phasic increases in EMGgg were associated with the resumption of flow, as previously described (15, 32–34, 38, 39). Decreases in Pn were associated with the development of inspiratory airflow limitation as defined by an early plateau in inspiratory airflow as Ptrach (and oropharyngeal pressure) continued to decrease (9, 29), indicating that the flow limiting site remained rostral to the palatal rim during inspiration. Metrics of active upper airway function were utilized to characterize the susceptibility to inspiratory flow limitation (IFL) and severity of IFL during negative pressure ramps, as described below.

Prior to each ramp decrease in nasal pressure, the tracheal cannula was flushed for 1 to 2 s. This flush led to an increase in lung volume, which elicited a pronounced central apnea. As tracheal pressure declined following the flush, several deep tidal breaths resumed and were associated with reproducible maxima in phasic EMG, as previously described (29).

Data Analysis

Each Pn ramp (run) was evaluated to determine the passive Pcrit and the presence and Pn onset of inspiratory airflow limitation as

<table>
<thead>
<tr>
<th>n</th>
<th>Age, wk</th>
<th>Weight, g</th>
<th>Normalized Min. Ventilation, μl·min⁻¹·g⁻¹</th>
<th>Tidal Volume, μl</th>
<th>Respiratory Rate, breaths/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J</td>
<td>Lean</td>
<td>10</td>
<td>9.2 ± 0.4</td>
<td>23.3 ± 0.4</td>
<td>6.0 ± 0.5</td>
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<tr>
<td>Obese</td>
<td>10</td>
<td>9.1 ± 0.2</td>
<td>29.5 ± 0.2</td>
<td>7.1 ± 0.6</td>
<td>242.7 ± 19.6</td>
</tr>
<tr>
<td>Ob/+</td>
<td>Lean</td>
<td>10</td>
<td>9.0 ± 0.2</td>
<td>23.9 ± 0.1</td>
<td>4.5 ± 0.3</td>
</tr>
<tr>
<td>Obese</td>
<td>9</td>
<td>8.9 ± 0.1</td>
<td>29.9 ± 0.2</td>
<td>4.8 ± 0.3</td>
<td>161.4 ± 10.2*</td>
</tr>
<tr>
<td>Very obese</td>
<td>8</td>
<td>8.8 ± 0.1</td>
<td>41.2 ± 0.7</td>
<td>5.8 ± 0.4</td>
<td>140.0 ± 9.2*</td>
</tr>
<tr>
<td>Leptin treated</td>
<td>8</td>
<td>8.9 ± 0.1</td>
<td>40.7 ± 0.6</td>
<td>7.6 ± 0.5‡</td>
<td>188.7 ± 14.4</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE. Baseline characteristics are shown for lean, obese, and very obese C57BL/6J (BL6) and ob+/ob+ mice that were matched by age and weight. Baseline ventilatory parameters are also shown for strain and weight groups. Normalized min. ventilation, minute ventilation (μl/min) divided by body weight (g). Significant increases in minute ventilation were observed with increasing weight, independent of strain (P < 0.001). Minute ventilation was lower in the ob+/ob+ murine group at each weight level (P < 0.001). A significant decrease in normalized ventilation was seen in the ob+/ob+ mice with increasing weight (P = 0.004). Acute leptin replacement increased minute ventilation and tidal volume compared with very obese ob+/ob+ mice (P < 0.001 and P < 0.0001, respectively).
follows. IFL frequency was defined as the percentage of PN ramps displaying inspiratory flow limitation. IFL threshold was defined as the PN at which inspiratory flow limitation commenced. This threshold was also referenced to the passive PCRIT level (PN – PCRIT) to account for differences in passive PCRIT (pharyngeal mechanical loads) between murine groups. The severity of airflow obstruction (IFL severity) was defined by the level of VImax at the onset of flow limitation.

For the first flow-limited breath of each run, the tonic and peak phasic EMG activity was measured. Phasic EMG was calculated as the difference between tonic and peak phasic levels. All EMG measurements were normalized to the maximal EMG in each mouse and expressed as a percentage of maximal activity, as previously described (29).

To assess for differences in ventilation among the groups, ventilation was measured during periods of stable breathing at baseline prior to nasal pressure ramps, and normalized by weight for each mouse (Table 1).

Statistical Analysis

Statistical analyses were structured to test a priori hypotheses that upper airway function varied as a function of murine strain (ob/ob vs. BL6) and weight (lean, obese, and very obese groups) and that leptin replacement restored upper airway function in ob/ob mice. Mixed effects linear regression was utilized to model effects of the primary independent variables (strain, weight group, and treatment) on outcome measures of ventilation and upper airway function while accounting for random effects among mice, in which parameters of upper airway function were assessed repeatedly (Intercooled Stata 9.2, Statacorp, College Station, TX). Our models incorporated terms to determine both the independent and interactive effects of these predictors on the passive (expiratory) PCRIT, inspiratory parameters of active upper airway function, and absolute and normalized levels of ventilation. When significant differences were detected, post hoc comparisons were performed (with Bonferroni correction) to determine the source of these differences. Statistical significance was inferred at a P < 0.05 level. Values were expressed as means ± SE.

RESULTS

Effect of Strain, Weight, and Leptin Replacement on Passive PCRIT

The effects of weight, strain, and leptin replacement on passive PCRIT are illustrated in Fig. 1. Passive PCRIT increased significantly with weight, independent of strain, and was elevated in the ob/ob compared with BL6 mice, independent of weight. Moreover, progressive increases in passive PCRIT were observed with weight in the ob/ob strain. Leptin replacement did not alter the passive PCRIT significantly from the level observed in very obese ob/ob mice.

Effect of Strain, Weight, and Leptin Replacement on the Phasic Modulation of Upper Airway Function

As expected, baseline levels of ventilation increased significantly with weight (Table 1). Ventilation was also significantly lower in the ob/ob compared with BL6 mice, independent of weight (22). After normalizing ventilation to body weight, however, we did not detect any significant differences in ventilation in the BL6 group but did see a significant decrease with weight in the ob/ob group, consistent with respiratory depression and increased body fat in ob/ob compared with BL6 mice (4). Nevertheless, acute leptin replacement increased minute ventilation and tidal volume compared with very obese ob/ob mice. Minute ventilation and tidal volume were restored to levels observed in the obese BL6 murine group, consistent with an immediate increase in CO2 production and/or increased ventilatory drive, independent of body composition (22).

During PN ramps, ob/ob mice showed a greater IFL frequency than BL6 mice across all weight groups (Fig. 2). The frequency of flow-limited inspirations declined significantly with increasing weight, independent of strain. When responses were assessed separately in each strain, we found that a weight-related decrease in IFL frequency was only observed in the BL6 but not ob/ob mice. Leptin replacement, however, produced a significant decrease in the frequency of flow-limited inspirations (~30%) in the very obese ob/ob mice, approaching levels found in the obese BL6 mice.

The effects of strain, weight and leptin replacement on PN thresholds are illustrated in Fig. 3, in which the absolute (PN) and relative PN (PN – PCRIT) thresholds to the development of IFL appear in the left and right panels, respectively. A significant increase in both thresholds occurred with weight, independent of strain, indicating that flow limitation developed at higher (less negative) levels of nasal pressure in ob/ob compared with lean mice. These thresholds were also significantly higher in ob/ob mice compared with BL6 mice across all weight groups. Of note, effects of weight and strain persisted even after referencing the PN threshold to the passive PCRIT (Fig. 3, right), suggesting that the elevation in PN threshold in leptin deficient, obese mice was independent of pharyngeal mechanical loads. Leptin replacement significantly decreased

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**Fig. 1.** Passive critical pressure (PCRIT) is illustrated by weight group for BL6 and ob/ob mice (mean ± SE). Passive PCRIT increased progressively with increasing body weight, independent of strain (*P < 0.001). In addition, passive PCRIT was higher (less negative) in the ob/ob group (*P < 0.001) than in the BL6 group. Passive PCRIT increased significantly with weight in the ob/ob mice (±P < 0.001). Leptin treatment (Leptin*) did not alter passive PCRIT significantly from the very obese leptin-deficient ob/ob mice.
We found that both obesity and leptin deficiency were associated with elevations in passive $P_{\text{CRT}}$. Compared with the BL6 mice, the $ob^{-}/ob^{-}$ mice also exhibited marked elevations in IFL frequency and severity, as well as elevations in the $P_N$ threshold at IFL onset, suggesting blunted neuromuscular responses to airway obstruction. Finally, leptin replacement decreased the IFL frequency, $P_N$ threshold and severity, suggesting that leptin replacement reversed defects in active neuromuscular responses without having any significant impact on passive $P_{\text{CRT}}$. These findings suggest that leptin can prevent the development of upper airway obstruction through a combination of peripheral mechanical and central neuromuscular actions. Leptin also increased minute ventilation, suggesting that its central effect in this leptin-deficient model may be attributable to a generalized increase in ventilatory drive (22).

Both obesity and leptin deficiency were associated with elevations in passive $P_{\text{CRT}}$ (3, 7, 21), reflecting increases in pharyngeal collapsibility in our mouse model. This effect parallels findings in sleeping humans demonstrating similar elevations in passive $P_{\text{CRT}}$ in obese compared with lean subjects (16). These effects have been attributed to fatty deposits in peripharyngeal tissues (35) and/or decreases in lung volume (7, 12, 24, 41) and concomitant decreases in caudal tracheal traction (44). We extend these findings in our mouse model by demonstrating that leptin deficiency, independent of obesity, leads to further elevations in pharyngeal collapsibility. This effect is best explained by alterations in body composition in $ob^{-}/ob^{-}$ mice, which exhibit marked increases in body fat compared with BL6 wild-type controls of similar body weight (4). In addition, $ob^{-}/ob^{-}$ mice also demonstrate reductions in lung compliance and lung volume (42). Resulting decreases in caudal tracheal traction would produce further increases in pharyngeal collapsibility (41). Thus combined effects of obesity and leptin deficiency on pharyngeal and lung mechanics can account for increases in upper airway collapsibility in our mouse model.

Leptin deficiency was also associated with marked decreases in active pharyngeal neuromuscular responses. In contrast to BL6 controls, the $ob^{-}/ob^{-}$ mice demonstrated marked increases in the frequency, $P_N$ threshold, and severity of IFL that reversed following leptin replacement. Although the frequency of IFL decreased markedly in obese wild-type mice, it remained elevated in $ob^{-}/ob^{-}$ mice, which demonstrated further increases in IFL $P_N$ threshold and severity with weight gain. These findings suggest that leptin prevents the development of IFL and protects against IFL in obesity. Leptin restores these active responses acutely, providing further evidence that leptin’s stimulation of neuromuscular responses can compensate for mechanical loads of obesity on the upper airway.

Obesity and leptin deficiency have been associated with decreased phasic dilation of the pharynx in obese compared with lean Zucker rats (3, 20). It is not known, however, whether this defect is due to loss of central drive or impaired mechanics of the muscles. Several lines of evidence suggest that active neuromuscular responses are depressed in $ob^{-}/ob^{-}$ mice and that leptin acts centrally to restore these responses. First, leptin increased ventilation acutely in our mice, consistent with an increase in ventilatory drive (22). Second, leptin has been demonstrated to increase hypercapnic ventilatory responses in conscious mice during sleep and wakefulness (22, 30). This increase was not related to increases in $CO_2$ production or alveolar ventilation, suggesting a direct effect on central hypercapnic responses (22). Finally, experimental evidence suggests that leptin sensitive regions of the nucleus tractus solitarius increase pharyngeal and phrenic neuromotor output under hypercapnic conditions (13, 14), particularly during periods of airway obstruction when inhibitory phasic volume feedback is removed (17, 39, 45). Thus our findings support current evidence that leptin acts centrally to increase active neuromuscular responses acutely and that leptin exerts its effect by increasing ventilatory drive rather than facilitating pharyngeal motor neuron activity directly.
Although our findings suggest both peripheral and central effects of leptin on upper airway patency, several limitations should be considered in interpreting these findings. First, measurements of passive $P_{\text{CRT}}$ provide estimates of the effect of structural/anatomic factors on pharyngeal collapsibility during expiration when neuromuscular activity falls to tonic levels. Although tonic EMG activity could decrease pharyngeal collapsibility (passive $P_{\text{CRT}}$), we previously demonstrated that neuromuscular blockade did not influence measurements of passive $P_{\text{CRT}}$ in this mouse model (29). Second, we acknowledge that anesthesia may have had differential effects on the strain and weight groups and may have attenuated responses in adipose-laden $\text{ob}^/-$/ob$^-$ mice. To account for differential effects of anesthesia on ventilation, we assiduously titrated isoflurane to maintain the breathing frequency in a narrow range. We found that ventilation normalized to weight actually declined as weight rose in $\text{ob}^/-$/ob$^-$ mice, which would in-

Table 2. EMGGG activity by strain and weight group

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Tonic</th>
<th>Peak Phasic</th>
<th>Phasic</th>
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</thead>
<tbody>
<tr>
<td>C57BL/6J</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean</td>
<td>8</td>
<td>11.8 ± 2.2</td>
<td>21.4 ± 4.7</td>
<td>11.1 ± 3.0</td>
</tr>
<tr>
<td>Obese</td>
<td>5</td>
<td>4.2 ± 0.8</td>
<td>24.8 ± 6.7</td>
<td>20.6 ± 6.1</td>
</tr>
<tr>
<td>$\text{ob}^/-$</td>
<td>10</td>
<td>9.3 ± 1.1</td>
<td>22.6 ± 2.8</td>
<td>13.3 ± 2.1</td>
</tr>
<tr>
<td>Obese</td>
<td>6</td>
<td>13.0 ± 1.6</td>
<td>23.6 ± 3.0</td>
<td>12.0 ± 2.1</td>
</tr>
<tr>
<td>Very obese</td>
<td>8</td>
<td>7.6 ± 3.1</td>
<td>22.4 ± 3.0</td>
<td>14.8 ± 2.8</td>
</tr>
<tr>
<td>Leptin treated</td>
<td>5</td>
<td>7.8 ± 2.7</td>
<td>30.0 ± 4.0†</td>
<td>22.2 ± 2.9†</td>
</tr>
</tbody>
</table>

Tonic, peak phasic, and phasic genioglossus electromyographic (EMGGG) measurements are shown for the first flow-limited breath of each run and expressed as means ± SE in % of maximum. Phasic EMGGG was calculated as the difference between tonic and peak phasic levels. EMGGG measurements were normalized to the maximal EMGGG in each mouse and expressed as a percentage of maximal activity. Leptin replacement significantly increased the peak phasic EMGGG and the phasic component (†P < 0.01) of the EMGGG compared with very obese $\text{ob}^/-$/ob$^-$ control mice of similar weight. In contrast, tonic, peak phasic, and phasic EMGGG did not differ significantly across strain (C57BL/6J vs. $\text{ob}^/-$/ob$^-$) or weight groups; nor did leptin replacement influence tonic EMGGG significantly in very obese $\text{ob}^/-$/ob$^-$ compared with $\text{ob}^/-$/ob$^-$ control mice of similar weight.

Fig. 3. Nasal pressure ($P_N$; left) and $P_N$ referenced to passive $P_{\text{CRT}}$ ($P_N - P_{\text{CRT}}$; right) thresholds to the development of flow limitation during negative pressure ramps are illustrated by weight group for BL6 and $\text{ob}^/-$/ob$^-$ mice (mean ± SE). $P_N$ threshold increased progressively with increases in body weight, independent of strain (‡P < 0.001). The $P_N$ threshold was also significantly elevated in the $\text{ob}^/-$/ob$^-$ compared with BL6 mice independent of weight (*P < 0.001). Leptin treatment lowered the $P_N$ threshold compared with the very obese leptin-deficient $\text{ob}^/-$/ob$^-$ mice (#P < 0.001). $P_N - P_{\text{CRT}}$ threshold also increased progressively with increases in weight, independent of strain (+P = 0.002). The $P_N - P_{\text{CRT}}$ threshold was also significantly elevated in the $\text{ob}^/-$/ob$^-$ compared with BL6 mice, independent of body weight (‡P < 0.001). Leptin treatment (Leptin*) lowered the $P_N - P_{\text{CRT}}$ threshold compared with the very obese leptin-deficient $\text{ob}^/-$/ob$^-$ mice (#P < 0.001).

Fig. 4. Maximum inspiratory airflow ($V_{\text{max}}$) during inspiratory flow limitation is illustrated by weight group for BL6 and $\text{ob}^/-$/ob$^-$ mice (mean ± SE). $V_{\text{max}}$ was higher in the BL6 group than in the $\text{ob}^/-$/ob$^-$ group, independent of weight (*P < 0.01). Additionally, $V_{\text{max}}$ decreased significantly with weight in the $\text{ob}^/-$/ob$^-$ group (‡P < 0.001). Leptin treatment (Leptin*) increased the $V_{\text{max}}$ compared with the very obese leptin-deficient $\text{ob}^/-$/ob$^-$ mice (#P < 0.001).
crease CO₂ and theoretically stimulate rather than decrease pharyngeal neuromuscular responses. Moreover, in leptin-treated mice, ventilation and active responses recovered without alterations in body weight or composition, suggesting that anesthesia did not confound our assessment of active upper airway function. Third, we restricted our studies to male mice to reduce sex-related variability in upper airway function, so our results cannot be generalized to female mice. Finally, differences in active EMG responses were not observed between ob⁻/⁻ and BL6 mice, possibly owing to technical factors interfering with EMG signal acquisition and/or that the genioglossus is one of several muscles controlling the upper airway. Nevertheless, EMG activity increased after leptin administration, consistent with a neuromuscular mechanism for the response. We also acknowledge that EMG activity can only reflect relative rather than absolute neuromuscular responses to negative airway pressure challenges, which limits inferences drawn about strain, weight, and leptin treatment effects on neuromuscular control. Further insight could be gained by assessing single motoneuron rather than integrated EMG responses in this model. Nevertheless, striking differences in active IFL responses were observed between strains that were largely abolished acutely by leptin replacement.

Our findings have implications for sleep apnea pathogenesis and treatment. Current evidence supports a two-hit mechanism for sleep apnea pathogenesis in which anatomic susceptibility to airway obstruction becomes manifest only in the presence of a concomitant defect in pharyngeal neuromuscular control (19, 26). The findings in our murine model suggest that leptin can mitigate upper airway mechanical loads and stimulate compensatory neuromuscular responses. Realizing leptin’s therapeutic potential in sleep apnea patients, however, may be challenging because human obesity and sleep apnea have been associated with elevations in circulating leptin concentrations (25, 40, 43), leading investigators to postulate a state of leptin resistance and/or CNS leptin insufficiency (6, 10, 18). Leptin resistance has also been associated with metabolic dysregulation, which can be ameliorated by CPAP treatment of sleep apnea without concomitant alterations in body weight or composition (1). These findings suggest that sleep apnea treatment can sensitize peripheral tissues to leptin (2), which could ultimately act to reduce fat deposition around the pharynx and decrease pharyngeal mechanical loads. In addition, limitations to leptin transport across the blood-brain barrier in obesity may reduce compensatory CNS neurostimulatory effects (5). Under these circumstances, its effect on ventilatory control could theoretically be augmented by agents that facilitate its transport into the CSF and/or sensitize the CNS to its activity (27, 28).

Leptin’s ability to enhance pharyngeal neuromuscular control may be related to a generalized (nonspecific) increase in ventilatory drive rather than direct stimulation of phrenic and upper airway motoneuron pools. Further research will be required to delineate leptin’s underlying mechanisms of action and discover agents that enhance its activity in pharyngeal and neural tissues.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


