Intermittent hypoxia reduces upper airway stability in lean but not obese Zucker rats

Andrew D. Ray,1,2 Ulysses J. Magalang,3,4 Charles P. Michlin,1 Toshiyuki Ogasa,3 John A. Krasney,2 Luc E. Gosselin,1 and Gaspar A. Farkas1,3

Departments of 1Exercise and Nutrition Sciences, 2Physiology and Biophysics, and 3Medicine, Division of Pulmonary, Critical Care and Sleep Medicine, State University of New York at Buffalo, Buffalo, New York; and 4Department of Medicine, Division of Pulmonary, Critical Care and Sleep Medicine, The Ohio State University, Columbus, Ohio

Submitted 19 January 2007; accepted in final form 24 April 2007

Ray AD, Magalang UJ, Michlin CP, Ogasa T, Krasney JA, Gosselin LE, Farkas GA. Intermittent hypoxia reduces upper airway stability in lean but not obese Zucker rats. Am J Physiol Regul Integr Comp Physiol 293: R372–R378, 2007. First published April 25, 2007; doi:10.1152/ajpregu.00038.2007.—Obstructive sleep apnea involves intermittent periods of airway occlusions that lead to repetitive oxygen desaturations. Exposure to chronic intermittent hypoxia (IH) in rats increases diurnal blood pressure and alters skeletal muscle physiology. The impact of IH on upper airway muscle function is unknown. We hypothesize that IH exposure increases upper airway collapsibility in rats due to alterations of the muscles surrounding the upper airway. Lean and obese rats were exposed to cyclic alterations in O2 levels (20.6%-5%) every 90 s, 8 h/day for 6 days/wk for 12 wk. Following the exposure period, arterial pressure was recorded via the tail artery in conscious unrestrained rats. Mean arterial pressure was measured under anesthesia during baseline conditions and then during supramaximal stimulation of the hypoglossal nerve (cnXII). Baseline Pcrit was more positive (more collapsible) in lean but not obese IH-exposed Zucker rats (P < 0.05). The pharyngeal pressure associated with airway collapse (Pcrit) was measured under anesthesia during baseline conditions and then during supramaximal stimulation of the hypoglossal nerve (cnXII). Baseline Pcrit was more positive (more collapsible) in lean but not obese rats following 12 wk of IH (P < 0.05), while supramaximal stimulation of cnXII increased airway stability (decreased Pcrit) in both lean and obese Zucker rats following IH to levels that were similar to their respective room air controls. The in vitro peak tension and the expression of the individual myosin heavy chain isoforms from the upper airway muscles were unaltered following IH. We conclude that IH leads to increases in baseline collapsibility in lean Zucker rats exposed to IH by nonmyogenic mechanisms.

A DECREASE IN UPPER AIRWAY muscle function promotes an unstable upper airway that is more susceptible to collapse (28). In obstructive sleep apnea (OSA), repetitive narrowing or collapse of the upper airway during sleep results in cyclic periods of intermittent hypoxia. Although the effects of chronic intermittent hypoxia (IH) have been implicated in the cardiovascular consequences of obstructive sleep apnea (7), IH’s effects on the structure and functional characteristics of the upper airway muscles are less well known. Because the upper airway muscles serve as the primary stabilizers of the upper airway, it is important to explore how IH impacts their ability to maintain airway patency.

Chronic exposure to continuous hypoxia has been shown to alter the structure and function of skeletal muscle. Bigard et al. (1) demonstrated that 12 wk of continuous hypoxia increases intermediate, type IIa and IIb fibers in both the extensor digitorum longus and plantaris muscles in rats. There is reason to believe that exposure to IH may also alter the structure of the upper airway muscles. It has been noted in animals with sleep-disordered breathing (27) and in humans with OSA that pharyngeal myopathy exists (38). However, the cross-sectional nature of the latter studies limits conclusions about causality.

Multiple authors have tried to understand the impact of IH on upper airway muscle structure; however, the results have been conflicting. McGuire et al. failed to detect any changes in upper airway muscle (geniohyoid and sternohyoid) composition or force production following 5 wk of IH in rats, although both muscles revealed increased fatigability (20). However, others have shown a slow to fast fiber transformation within hours of IH exposure in the geniohyoid and sternohyoid muscles, albeit in the absence of altered force production (25). Thus, it remains to be determined whether the increase in airway collapse is related to a myogenic dysfunction as a result of intermittent exposure to hypoxia. Therefore, the following study will address the functional impact of IH-induced structural alterations on upper airway muscle and their ability to maintain airway stability.

Obesity is known to impact the size and function of the upper airway. Indeed, a significant number of patients with OSA are obese (36). The obese Zucker rat (fa/fa), a genetic model of early onset obesity has a defective leptin receptor (13) and develops hyperleptinemia, hyperphagia, and hyperinsulinemia (2). Obese Zucker rats present with many of the same respiratory deficits commonly observed in obese humans, including reduced lung volumes, reduced chest wall compliance, a blunted hypoxic ventilatory response, an anatomically altered upper airway (3, 4, 18), and most importantly, a more collapsible upper airway compared with their lean counterparts (21, 24). On the basis of the multiple similarities to obese humans, exposure of the obese Zucker rat to IH may be more representative of the situation in humans with OSA.

The goals of the current study were 1) to investigate the impact of IH exposure on the functional, mechanical, and biochemical characteristics of the upper airway muscles, and 2) to determine whether obese animals with an already compromised upper airway respond in a similar manner. We hypothesized that IH would reduce upper airway stability and that the changes would be directly attributed to alterations in...
the upper airway muscles. This study utilized a functional approach to assess the impact of IH on the ability of the upper airway muscles to maintain airway patency.

METHODS

Animals. The experiments were initially carried out in 18 lean and then in 16 obese age-matched (11–13 mo) male Zucker rats purchased from Vassar College. The Institutional Animal Care and Use Committee at the University of Buffalo approved all protocols. Food and water were available ad libitum.

Intermittent hypoxia. Room air (RA) control and IH-exposed animals from both strains (lean and obese) were transferred to smaller cages during the exposure period. IH exposure consisted of 90 s of progressive hypoxia, followed by a return to RA over a 90-s period, 8 h/day, 6 days/wk for 12 wk. Decreases in $F_{\text{I}}$O$_2$ levels were achieved by delivering 100% nitrogen at an appropriate flow rate to lower the oxygen content in each cage to 4–5% for 3–5 s. This was immediately followed by a switch to RA oxygen levels prior to the next hypoxia exposure. At the end of each daily exposure period, rats were returned to their home cages. Food and water were available ad libitum at all times.

Blood pressure recordings. Following the 12-wk protocol and within 12–18 h after the last hypoxia exposure, rats were anesthetized with isoflurane and chronically catheterized via the tail artery. A polyethylene catheter (OD = 0.80 mm, ID = 0.60 mm) was inserted via the tail artery and advanced to the level of the abdominal aorta. The catheter was tunneled subcutaneously to exit the posterior nape of the neck and attached to a swivel (Instech Solomen Labs, Plymouth Meeting, PA). Arterial blood pressure recordings were obtained in awake, freely moving rats continuously over a 4-h period (between 0800 and 1200 noon) one day following arterial catheterization. A data acquisition program was used for real-time recording and later analysis of the blood pressure response (Dataq Instruments, Akron, OH).

Upper airway mechanics. An isolated upper airway preparation previously used in our laboratory was applied to determine the pharyngeal critical pressure ($P_{\text{c}}$), maximal inspiratory airflow ($V_{\text{Imax}}$), and oro-nasal resistance (Ron) (21, 24). A more detailed description of the methods is provided by McGuire et al. (20). Briefly, rats were mechanically ventilated (Model 683; Harvard Apparatus, South Natick, MA) and anesthetized with 4% isoflurane; once a surgical plane of anesthesia was reached the percentage of isoflurane was titrated down between 2–3%. End-tidal CO$_2$ was monitored continuously (Capstar-100; CWE, Ardmore, PA) through the caudal tracheal stab and maintained at 5% by adjusting the rate of the mechanical ventilator. A rigid cannula was placed in the rostral cut end of the trachea and tied into place. A mobile catheter with a side second catheter was inserted into the rigid cannula to record the hypopharyngeal (Php) pressure and was positioned lower in the airway. A negative pressure source was supplied downstream from the airway to identify the flow limiting site. Php, Php, and blood pressure were monitored by pressure transducers (P23XL; Statham Laboratories, Oxnard, CA). The inspiratory airflow through the airway was measured with a pneumotachograph (No. 0.771; Fleisch, Switzerland) and a differential pressure transducer (Model MP 45–1, range ± 2 cmH$_2$O, Validyne Engineering, Northridge, CA).

To determine the ability of the upper airway muscles to maintain upper airway stability, we measured upper airway mechanics during electrical stimulation of hypoglossal nerve (cnXII). Indeed, we acknowledge that the diameter of the upper airway is under the influence of multiple motor units; however, cnXII and the genioglossus muscle have been shown to be the primary upper airway dilator (23). The common branch of cnXII (bilateral) was dissected free from where it crossed the carotid artery and was sectioned just proximal to the bifurcation into its lateral and medial branches. Mineral oil was applied to prevent nerve desiccation. Stimulating electrodes were connected to a constant current electrical stimulus isolation unit (Model SIU8T; Grass Instruments, Quincy, MA) and stimulator (S44 Stimulator; Grass Instruments). Fifteen to twenty minutes following cnXII denervation, $P_{\text{crit}}$, $V_{\text{Imax}}$, and Ron were measured during bilateral cnXII stimulation (pulse duration of 0.1 μs, stimulation frequency of 100 Hz, and stimulation voltage of 40 V). We acknowledge testing the muscles at this level of stimulation intensity represents maximal activation and that during respiration the level of muscle activity could vary and/or be much less. An average of three measurements of $P_{\text{crit}}$, $V_{\text{Imax}}$, and Ron were obtained under two separate conditions: 1) unstimulated and 2) during cnXII stimulation.

Contractile properties of the upper airway and chest wall musculature. In vitro contractile properties of the sternohyoid were measured as previously described (5). Briefly, portions of the muscle were carefully removed, dissected into smaller bundles, and immersed in an oxygenated Krebs solution (37°C). Following a thermo-equilibration period (10 min), the muscle bundle was adjusted to its optimal length ($L_0$), and maximal twitch tension was measured. Five twitches were recorded at $L_0$, and the average value was used to determine the time to peak tension (TPT), half-relaxation time (RT1/2), and peak twitch force ($P_{\text{t}}$). TPT was defined as the time from the start of contraction to the point at which peak tension was achieved. RT1/2 is defined as the TPT to the point where peak tension dropped by 50%. Maximal tetanic force ($P_{\text{t}}$) was calculated and expressed in Newtons of force per square centimeter of muscle tissue. Muscle cross-sectional area was approximated by dividing the muscle mass by its length and muscle density (1.056 g/cm$^3$). Fatigability of the muscles was assessed by observing the loss of force in response to repeated stimulations over a 2-min period. The muscle was stimulated once per second with 350-ms trains at a frequency of 35 Hz. On completion of the measurements, the muscle bundle was removed from the apparatus, blotted dry, and weighed. Force was recorded at 10-s intervals and expressed as a percentage of the initial force.

Myosin heavy chain analysis. Myosin heavy chain (MHC) isoforms from the genioglossus and sternohyoid muscles were separated using SDS-PAGE using a mini-gel system (Bio-Rad, Hercules, CA). The gels consisted of a 4% acrylamide (30% glycerol, pH 6.8) stacking gel and an 8% acrylamide resolving gel (30% glycerol, pH 8.8). The samples were run at a constant voltage (150 V) for 20–24 h at 5°C. Following electrophoresis, the gels were silver-stained according to a technique by Oakley et al. (22). A digital image was then taken of the gel and the relative distribution of each isoform (I, 2A, X, and 2B) was determined by densitometry as previously described (10).

Statistical analysis. The differences in upper airway mechanics, the expression of the myosin heavy chain isoforms, and the in vitro fatigue curves were analyzed with a two-way ANOVA with repeated measures. The between-subject factors were RA vs. IH for all measurements, the within-subject factors were base vs. stimulation (upper airway mechanics), the expression of the four different MHC isoforms, or the time to fatigue. If statistical significance existed, a post hoc t-test with Bonferroni correction was used to determine the differences. A two-way ANOVA was performed on airway collapsibility between lean and obese animals during baseline (nerve intact) and during cnXII stimulation. Blood pressure and P$_{\text{t}}$ were analyzed with t-tests. A value of $P < 0.05$ was considered significant. All data are presented as means ± SE.

RESULTS

Body weight. No difference in body weights was observed between lean room air (Ln-RA) and lean intermittent hypoxia (Ln-IH) groups at the beginning [479 ± 13 g vs. 501 ± 19 g, respectively, not significant (NS)] or at the end of the 12-wk exposure period (513 ± 13 g vs. 506 ± 16 g, respectively, NS). Despite no differences in body weight at the beginning or at the
end of the study, Ln-RA rats gained significantly more weight during the 12-wk exposure period compared with the Ln-IH rats.

Obese rats weighed more than their lean age-matched counterparts at all stages of the study. Although body weights did not differ between obese groups at the beginning of the experimental period (701 ± 7 g vs. 687 ± 14 g, obese room air (Ob-RA) and obese intermittent hypoxia (Ob-IH), respectively, NS], Ob-RA rats weighed significantly more following the 12-wk experimental period compared with the Ob-IH rats (752 ± 21 g vs. 710 ± 21, P < 0.05).

Blood pressure. Mean arterial pressure (109 ± 14 vs. 96 ± 13 mmHg, P < 0.05) and systolic blood pressure (126 ± 16 vs. 112 ± 14 mmHg, P < 0.05) were significantly elevated in Ln-IH rats compared with Ln-RA rats, respectively. However, diastolic blood pressures (73 ± 7 vs. 377 ± 8.7 beats/min) were not different between Ln-RA and Ln-IH rats, respectively. In marked contrast, mean arterial blood pressure was not elevated in Ob-IH rats compared with the Ob-RA rats (101 ± 12 mmHg vs. 99 ± 17, respectively, NS).

Upper airway mechanics. Upper airway dynamics were recorded in 10 Ln-RA and 8 Ln-IH rats. Excessive bleeding during the isolated upper airway preparation prevented data collection from two Ln-IH rats. Unstimulated upper airway collapsibility (Pcrit) was significantly increased (more positive, more collapsible) in lean control rats following IH compared with lean control rats (Fig. 1, left). Despite the change in unstimulated collapsibility, Ron and VImax through the upper airway were not altered following 12 wk of IH. Furthermore, cnXII stimulation significantly decreased Pcrit (more negative, more stable) and increased VImax to levels that were similar between both groups of lean rats (Fig. 1, left).

Upper airway dynamics were measured in eight Ob-RA and five Ob-IH animals (Fig. 1, right). Excessive bleeding and airway secretions during the procedure prevented airway collapsibility measurements in three Ob-IH rats. During unstimulated recordings, upper airway collapsibility and Ron were not altered in obese Zucker rats following IH; however, VImax was significantly elevated in the Ob-IH rats compared with the Ob-RA rats. As a result of cnXII stimulation, Pcrit became more negative (less collapsible) in both groups of obese rats compared with their respective unstimulated trials; however, Pcrit was more negative (more stable) in Ob-RA rats compared with Ob-IH rats. While cnXII stimulation increased VImax in both groups of rats, only the Ob-IH rats attained a significantly higher airflow.

To summarize, the upper airways of the Ob-RA rats became more stable during stimulation, but the increase in VImax was offset by an increase in Ron. Similarly, the Ob-IH rats became more stable during stimulation (Pcrit, more negative); however, VImax reached a higher level because Ron did not change.

As noted previously (21), the upper airways from the Ob-RA group are more collapsible compared with the Ln-RA group. Despite changes in upper airway collapsibility following IH in lean rats only, the Ob-IH rats remained more collapsible compared with the Ln-IH rats. Even during cnXII stimulation,
the obese upper airways remained more collapsible compared with their age-matched lean counterparts.

Contractile characteristics. The contractile properties from the sternohyoid muscle were studied following the 12-wk protocol in lean Zucker rats. Twitch and tetanic stimulation data are presented in Table 1. Compared with control rats, IH had no impact on Po, RT1/2, TPT, Pt, and the P/P0 ratio. Furthermore, the time course to fatigue induced by repetitive stimulation was not altered following IH (Fig. 2).

Contractile properties from the obese sternohyoid muscle are also presented in Table 1. Compared with control values, Po, RT1/2, TPT, Pt, and the P/P0 ratios were not different following IH. In addition, the time course to fatigue induced by repetitive stimulation was not altered in obese rats following IH (Fig. 2).

Myosin heavy chain isofrom composition. Myosin heavy chain (MHC) isoform analysis was performed on portions of the genioglossus and sternohyoid muscles from 14 lean Zucker rats (Ln-RA = 7, Ln-IH = 7). The expression of the individual MHC isoforms from both muscles is presented in Fig. 3. Following IH, only the IIA isoform from the sternohyoid muscle changed, whereas no differences were noted in the other three isoforms (types I, IIX, and IIB) or from the genioglossus muscle.

The individual MHC isoforms were also isolated from obese genioglossus and sternohyoid muscle (Ob-RA, n = 7; Ob-IH, n = 7) and displayed in Fig. 3. No significant differences were observed in the percentages of the four MHC isoforms (types I, IIA, IIX, and IIB) from either the genioglossus or sternohyoid muscles following IH exposure.

DISCUSSION

The primary findings from this study are 1) exposure to IH renders the upper airway more collapsible and raises arterial blood pressure in lean Zucker (LnZ) rats; 2) the increase in airway collapsibility is not associated with changes in the musculature that supports upper airway function, and 3) as we have reported previously (21, 24), upper airway stability is reduced in obese Zucker (ObZ) rats compared with LnZ rats, and the present study indicates that imposition of IH does not appear to further alter upper airway stability in ObZ rats. The results from the present study demonstrate a significant relationship between IH and the in vivo collapsibility of the isolated upper airway in lean, but not obese Zucker rats. This study is the first to demonstrate a relationship between IH and a functional measure of upper airway dynamics in any animal model.

Table 1. In vitro contractile properties from the sternohyoid muscle

<table>
<thead>
<tr>
<th></th>
<th>Po, N/cm²</th>
<th>TPT, ms</th>
<th>1/2 RT, ms</th>
<th>Pt, N/cm²</th>
<th>P/P0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ln-RA</td>
<td>11.6±1.4</td>
<td>15.7±0.7</td>
<td>11.1±0.8</td>
<td>1.5±0.2</td>
<td>0.25±0.01</td>
</tr>
<tr>
<td>Ln-IH</td>
<td>13.5±2.7</td>
<td>16.6±0.4</td>
<td>10.6±0.6</td>
<td>1.7±0.2</td>
<td>0.25±0.03</td>
</tr>
<tr>
<td>Ob-RA</td>
<td>15.1±1.9</td>
<td>16.8±0.5</td>
<td>12.1±0.7</td>
<td>1.4±0.5</td>
<td>0.24±0.1</td>
</tr>
<tr>
<td>Ob-IH</td>
<td>10.9±2.7</td>
<td>17.1±0.6</td>
<td>11.1±0.5</td>
<td>1.3±0.4</td>
<td>0.31±0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; Po, maximal tetanic force; TPT, time to peak tension; 1/2 RT, time it takes for peak tetanic force to decrease by 50%; Pt, peak twitch force; P/P0, ratio of the peak tetanic force to peak twitch force; Ln-RA, lean room air; Ln-IH, lean intermittent hypoxia; Ob-RA, obese room air; Ob-IH, obese intermittent hypoxia. P = not significant for all values.

Before discussing our results, several limitations of the isolated upper airway preparation should be addressed. First, it is well known that anesthesia depresses respiration and genioglossal muscle activity. However, previous work from our laboratory has shown that although activation of the genioglossus muscle is depressed, it is not completely eliminated in anesthetized preparations (21). To offset any possible limitations of anesthesia, the levels of anesthesia and end tidal CO2 (5%) were maintained relatively constant throughout the duration of the procedure. Second, large negative suction pressures were applied to define the point of airflow limitation using our isolated upper airway preparation. These large negative pressures are not physiological and could conceivably traumatize the upper airway, leading to alterations in airway collapsibility. However, our experience with the model has demonstrated that repeated measures of upper airway collapsibility are reproducible with little variation between trials. Lastly, the present study exposed animals to 72 days of IH compared with the 35 days reported in most other studies. Since data were not collected at intermittent time points during the 12-wk protocol, it is difficult to directly compare our findings with those from studies where animals were exposed for shorter periods of time.

OSA is characterized by repetitive collapse of the upper airway during sleep that is associated with arterial oxygen desaturation. The mechanisms leading to airway occlusion are multiple and interrelated. There is evidence that pharyngeal myopathy may contribute to the increase in airway collapsibil-
ity (26). Changes to upper airway muscle function have been reported in humans with OSA (32, 33, 38) and in the English bulldog, a naturally occurring model of OSA (27). These muscular changes may be the result of an increase in workload (11, 12) or secondary to the direct effects of hypoxia acting on skeletal muscle (1) or both. However, these studies do not establish whether IH contributes to the upper airway muscle dysfunction due to their cross-sectional design. The advent of the rodent model of IH eliminates the cross-sectional design and allows the disease to be studied over a significant portion of the animal’s life. Therefore, the current study was designed to determine whether IH leads to upper airway dysfunction and whether these changes are correlated with functional deficits manifest as an inability of the upper airway muscles to maintain airway stability.

The results from the current study demonstrate a detrimental effect of IH on the ability of the upper airway muscles to maintain airway stability in LnZ rats. More specifically, only LnZ rats exposed to IH demonstrated an elevated $P_{\text{crit}}$ (more positive, more collapsible) under unstimulated conditions. The specific mechanism accounting for the increase in upper airway collapsibility following IH is difficult to identify and could be caused by either myogenic (failure of the muscle) or neuronal (failure to recruit) mechanisms or a combination of both.

Stimulating cnXII is a method we have used to assess the integrity of the primary upper airway dilator, the genioglossus muscle. It also allows us to differentiate between myogenic vs. neuronal dysfunction of the upper airway muscles. We recognize that support to the upper airway is not mediated solely through cnXII and the genioglossus muscle. Other muscles are also known to dilate the upper airway and provide additional support (16, 31). Previously, we have demonstrated that a serotonergic agonist ($5\text{-HT}_{2a/c}$) is capable of improving upper airway mechanics in both control and cnXII denervated lean and obese Zucker rats, suggesting that serotonergic control of the upper airway is not mediated solely through the genioglossus but by other pharyngeal muscles as well (24). However, the improvements seen in the cnXII denervated rats was on a much smaller scale compared with the improvements observed in the control rats, suggesting that cnXII is the primary innervation to the upper airway dilators. These results are consistent with other studies suggesting the genioglossus is the primary upper airway dilator (23). From the current study, direct stimulation of cnXII reduced $P_{\text{crit}}$ (more stable) to levels that were below unstimulated conditions but that were similar between both groups of LnZ rats. As a result of a more stable upper airway during stimulation, airflow through the upper airway was increased, a response that is consistent with previous studies (21, 30). Thus, the ability of the upper airway muscles to stabilize the airway following IH was preserved, suggesting that the decrease in upper airway stability following IH is likely not related to primary alterations in myogenic function but rather is more likely due to alterations in neurological control of the upper airway muscles.

Further evidence, excluding myogenic dysfunction as a contributor to the increase in upper airway collapsibility, was provided by the in vitro contractile characteristics of the sternohyoid muscle. Exposure to IH did not alter peak force production from the sternohyoid muscle, a response that is consistent with past studies utilizing a similar approach (20). Although overall absolute force values tended to be low in both groups, no differences were noted between RA- or IH-exposed animals. The low force values may be attributed to manipulations of the sternohyoid during upper airway collapsibility tests or to the fact that sternohyoid muscles were cut at both ends (6). Nevertheless, all animals underwent an identical procedure, such that both groups would have been equally affected. In contrast to previous studies, we found no change in sternohyoid fatigue following IH. Although it is unclear why the current study failed to demonstrate an increase in fatigue, we suggest that the longer time of exposure may have impacted the functional and biochemical characteristics of the muscle differently than those studies using a shorter exposure period (20, 25).
Consistent with the above results, the expression of the individual myosin heavy chain isoforms isolated from the upper airway muscles was unaltered following IH. Although a slow to fast fiber transformation has been reported in limb muscles in rats exposed to continuous hypoxia (1), McGuire et al. (20) failed to detect any fiber type transformation from the upper airway muscles (geniohyoid and sternohyoid) following 35 days of IH in rats. In contrast, Pae et al. (25) demonstrated a slow to fast transformation in the MHC isoforms from the geniohyoid and sternohyoid following hours of exposure to IH (25). However, using the English bulldog as a natural model of OSA, Petrof et al. (27) suggested the characteristic slow to fast fiber transformation in the sternohyoid muscle is the result of an increase in the tonic and phasic activity of the upper airway muscles and not the consequence of hypoxia (27). From the current study, IH had no impact on the expression of the MHC isoforms from the geniohyoid; however, the sternohyoid muscle demonstrated a small but significant decrease in the Iia isoform, a change that is consistent with most skeletal muscle that has been exposed to continuous hypoxia. Despite a slight change in the sternohyoid MHC composition following IH, the decrease in the Iia isoform did not affect muscle contractility. The inability to alter the expression of the MHC isoforms is consistent with the inability to alter force production or fatigability in the sternohyoid muscle following IH.

Consistent with previous studies, LnZ rats demonstrated an increase in mean arterial pressure following IH (8). However, it is uncertain whether the changes in arterial blood pressure could independently contribute to the accompanying alterations in upper airway dynamics. An inverse correlation between baroreflex sensitivity and genioglossal muscle activity has been demonstrated, such that phenylephrine-induced increases in arterial pressure were associated with decreases in genioglossal muscle activity (9, 19) and increases in pharyngeal collapse (19). By contrast, however, phenylephrine has also been reported to increase vascular tone and decrease upper airway collapse (37). In spite of these conflicting findings, we have previously shown that elevations in blood pressure per se are not correlated with increases in upper airway collapse in Zucker rats (24). Therefore, it is unlikely that increases in blood pressure significantly contributed to the decrease in upper airway stability that we observed in LnZ rats following IH.

Recently, data have been presented supporting plasticity of the neurological control mechanism following IH that is consistent with a decrease in airway stability. Veasey and colleagues (35) demonstrated a decrease in serotonergic control of cnXII following 3 wk of IH in Sprague-Dawley rats. This decrease in serotonergic excitability of cnXII following IH was correlated with an increase in oxidative damage in the dorsal medial medulla (35). Therefore, it is possible that the hypoxia/reoxygenation cycles in LnZ rats resulted in central oxidative damage to serotonergic control of the hypoglossal nerve and/or its controlling nuclei, rendering the upper airway more collapsible.

**Impact of IH on upper airway** In contrast to the LnZ rats, upper airway collapsibility was not altered following IH in ObZ rats. Furthermore, cnXII stimulation decreased $P_{\text{crit}}$ to levels that were below unstimulated conditions and similar between both groups of obese rats, providing additional evidence that, when activated, the upper airway muscles are capable of producing sufficient force to increase upper airway stability, despite IH exposure. Consistent with the in situ results, the in vitro force production from the sternohyoid and the expression of the MHC isoforms from the sternohyoid and genioglossus muscles were not altered following IH in ObZ rats. Although, IH did not affect upper airway stability in ObZ rats, their upper airways were already more collapsible compared with their lean counterparts.

The increase in upper airway collapsibility observed in this study confirms our previous observations in ObZ rats (21, 24) and may be the result of an increase in body weight and/or altered neurological control of the upper airway muscles. ObZ rats differ from LnZ rats in that they also present with neurological abnormalities that predispose them to upper airway instability. ObZ rats have altered serotonergic activity that has been correlated with their overeating and obesity (34). Serotonin is also known to play an important role in controlling upper airway motor neurons across different sleep-wake states (14, 15). Previously, we have shown that obese, but not LnZ rats, rely more on their serotonergic system to maintain ventilation and airway stability (21). Moreover, administration of a serotonergic antagonist to LnZ has minimal effects on upper airway muscle activity, suggesting that, under normal physiological conditions, serotonergic drive to the hypoglossal motor nucleus is less in the lean compared with obese Zucker rats (29). As a result, upper airway dynamics in the ObZ rat were already maximally compromised before IH exposure and could not be further compromised.

The ability to sense hypoxia through the carotid bodies is a necessary step in the development of hypertension in rats exposed to IH (17). Unlike LnZ rats, exposure to IH did not increase mean arterial pressure in the ObZ rats. It has previously been shown that ObZ rats have a depressed hypoxic ventilatory response (4). Therefore, we suggest that a depressed hypoxic ventilatory response may have attenuated the IH-induced stimulation of the sympathetic nervous system and reduced the hypertensive response in the ObZ rat. Second, it is also possible that a hypoxic threshold needs to be reached to stimulate the cascade of events leading to diurnal hypertension, and it may be that the ObZ rats require a more severe desaturation to induce the same sympathetic response compared with their lean counterparts. However, lean and obese Zucker rats were exposed to similar levels of $F_{\text{IO}}$, during the exposure period, and thus we would predict that arterial oxygen desaturation would be more severe in the ObZ during exposure due to their reduced hypoxic ventilatory drive. Despite presumably more severe hypoxia, we failed to detect a chronic hypertensive response in the ObZ rats and can only speculate that a depressed arterial chemoreceptor sympathetic reflex limb may account for the failure to observe hypertension in the ObZ rats. Thus, we suggest future studies directed toward the cardiovascular response to IH in the ObZ rat are warranted to answer these questions.

In summary, the increase in upper airway collapsibility observed in LnZ rats following IH exposure is unlikely to be related to a decrease in the functional ability of the upper airway muscles to maintain upper airway stability. Rather, in light of the recent findings from Veasey et al. (35), the neurological control mechanisms to the upper airway muscles may be altered following IH. Therefore, we suggest future studies be directed toward examining the effects of IH on the
neural mechanisms controlling the upper airway muscles, with special attention to the serotonergic system.

ACKNOWLEDGMENTS

Present address of T. Ogasa: First Department of Internal Medicine, Asahikawa Medical College, 2-1-1-1 Midorigaoka-Higashi, Asahikawa, Hokkaido 078-8510, Japan.

GRANTS

This research was supported by grants from Research and Health in Erie County, The American Lung Association, New York Affiliate, and The Mark Diamond Research Grant, University at Buffalo.

REFERENCES