Upper airway loading induces growth retardation and change in local chondrocyte IGF-I expression is reversed by stimulation of GH release in juvenile rats

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Shraga Segal Department of Microbiology and Immunology, Faculty of Health Sciences, Ben-Gurion University of the Negev; Sleep-Wake Disorders Unit, Soroka University Medical Center; and Department of Physiology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel

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Segev Y, Berdugo-Boura N, Porati O, Tarasiuk A. Upper airway loading induces growth retardation and change in local chondrocyte IGF-I expression is reversed by stimulation of GH release in juvenile rats. J Appl Physiol 105: 1602–1609, 2008. First published September 11, 2008; doi:10.1152/japplphysiol.90772.2008.—Chronic resistive airway loading (CAL) impairs growth in juvenile rats. The effects of CAL on epiphyseal growth plate (EGP) structure and insulin-like growth factor (IGF)-I gene expression have not been explored. Little is known about whether stimulants of endogenous growth hormone (GH) secretion can normalize this growth impairment. This study explored the effect of CAL on circulating and EGP GH/IGF-I pathway GH and the effect of ritanserin (endogenous GH stimulant) on somatic growth and the GH/IGF-I axis. We hypothesized that CAL would lead to a decrease in body temperature (Tb) and alterations of Tb and locomotion activity (MA) despite preserved food consumption. CAL impaired both tibial and tail length gains. Tail and tibial length gains inversely correlated with tracheal resistance. Circulating GH and IGF-I, liver and EGP IGF-I mRNA, and EGP width were decreased in the CAL group. Ritanserin administration to CAL animals normalized circulating and local EGP GH and IGF-I levels and minimized the longitudinal growth impairment. We conclude that CAL causes growth delay associated with alterations in the GH/IGF-I axis. Stimulation of GH release by ritanserin restored both global and local GH/IGF-I pathways, yet growth parameters were only partially restored.

chronic resistive loading; epiphyseal growth plate; insulin-like growth factor I

ONE OF THE BETTER-KNOWN CONSEQUENCES of sleep-disordered breathing is the greater risk for failure to thrive (2, 6). Of all the pathways that can potentially explain longitudinal growth failure in sleep-disordered breathing, the growth hormone (GH) hypotheses have received the most attention (2, 4, 6, 8). The mechanisms leading to reduction of insulin-like growth factor (IGF)-I levels in children with sleep-disordered breathing are not clear. It has been suggested that the improvement of sleep following adenotonsillectomy (41, 45) is associated with enrichment of circulating IGF-I levels and growth after surgery (2, 48).

Chronic resistive airway loading (CAL) impairs longitudinal growth in prepubescent rats (44) and leads to failure to gain body weight in adult rats (13, 37, 43). This impaired longitudinal growth is due to impaired circulating GH and IGF-I levels (44), but the molecular-level changes at the epiphyseal growth plate (EGP) have not been explored. Impairment of GH secretion from the anterior pituitary somatotrophic cells and impairment of the liver GH/IGF-I pathway are involved in this impaired longitudinal growth (44). The liver is the main organ for IGF-I synthesis; other tissues in the periphery likely account for a smaller percentage of circulating levels as well as local production of IGF-I (47). The ultimate target organ is the EGP located on the long bones. IGF-I subsequently mediates GH action on the EGP through specific receptors (3, 5, 28).

Sleep impairment in rats can lead to a decrease in body weight gain and a decrease in body temperature (Tb) despite preserved or elevated energy intake (9, 33, 34). Sleep impairment can also lead to elimination of circulating GH and IGF-I levels compared with the prefragmentation baseline period (9). The effect of CAL on Tb and locomotion activity (MA) and EGP structural changes and IGF-I gene expression were not investigated in our CAL rat model (44). Therefore, we hypothesized that CAL would lead to a decrease in Tb and a reduction of serum GH and liver and EGP IGF-I levels, consequently leading to growth retardation.

Ritanserin, a selective 5-hydroxytryptamine-2 receptor antagonist, which has been shown to possess anxiolytic properties, is known to increase endogenous GH release (14). We hypothesized that ritanserin administration at sleep onset would promote increased endogenous GH release, consequently minimizing longitudinal growth impairment in CAL rats.

In the present study we explored the effect of CAL on Tb and MA, longitudinal growth, liver and EGP IGF-I gene expression, and EGP structural changes 14 days after surgery. We also explored whether pharmacological stimulation of endogenous GH release by ritanserin improves longitudinal growth and the GH/IGF-I axis.
MATERIALS AND METHODS

Animals. Our laboratory has previously used a surgical technique to induce CAL (12, 13, 37, 42–44) in 22-day-old prepuberty Sprague-Dawley male rats. For all surgical procedures, animals were anesthetized with tri bromoethanol (200 mg/kg ip). Sham surgery performed on the control group consisted of tracheal dissection without placement of a tracheal band. In the experimental group, increased tracheal resistance was imposed by a surgical technique for placement of a circumferential tracheal band. A midline ventral cervical incision was made, and the trachea was exposed and dissected so as not to damage adjacent structures. A 0.5-cm-long circumferential plastic band was placed around the trachea. A suture was looped around the band and tightened, thus constricting the trachea so as to increase inspiratory esophageal pressure swings two- to threefold. The skin incision was sutured, and animals were returned to their sound-attenuated cages (23 ± 1.0°C) for recovery and given food and water ad libitum, with a 12:12-h light-dark cycle (lights on at 0900).

The study was approved by the Ben-Gurion University of the Negev Animal Use and Care Committee and complied with American Physiological Society guidelines.

Body temperature and locomotion activity. Surgical preparation (23) consisted of shaving the abdomen and scrubbing the shaved area with Betadine and alcohol. A 0.5-cm incision was made through the skin and abdominal muscle layer with aseptic technique. A battery-operated free-floating transmitter (model TA10TA-F20, Data Sciences International, St. Paul, MN) was inserted into the abdominal cavity. The transmitter was able to move freely among the peritoneal organs because it was not attached to the peritoneum. The peritoneal muscle and skin layers were closed with interrupted sutures. Telemetric data were collected for 5 consecutive days starting from postsurgery day 9. Tb (±0.1°C) and MA were continuously monitored with the Dataquest A.R.T. system (Data Sciences International). The signal emitted by the transmitter is proportional to Tb. MA (counts) is obtained by counting the number of impulses, detected by changes in the level of the main carina to provide a continuous flow (Aalborg Instruments) of compressed air. Tracheal pressure was measured proximal to the circumferential tracheal band. Tracheal resistance was determined as the slope of flow-pressure curves in the linear portion of the curve (0.1–0.5 l/min). In a subset of five CAL and five control animals, arterial blood gases (pH, PCO2, PO2, and HCO3) were measured immediately after animal death (13). The trachea was exposed and cannulated with a catheter at the level of the main carina to provide a continuous flow (Aalborg Instruments) of compressed air. Tracheal pressure was measured proximal to the circumferential tracheal band. Tracheal resistance was determined as the slope of flow-pressure curves in the linear portion of the curve (0.1–0.5 l/min). In a subset of five CAL and five control animals, arterial blood gases (pH, PCO2, PO2, and HCO3) were determined 14 days after surgery (12, 13, 44).

Longitudinal growth. Somatic growth was measured before surgery and at day 14 of observation by measuring body weight and tibial and tail lengths with a digital caliper as previously described by our group (39, 43, 44). Two 5-µm hematoxylin and eosin-stained sections of bone EGP were obtained and analyzed by light microscopy (11) with approximated by esophageal pressure, was measured by means of a saline-filled catheter placed in the lower one-third of the esophagus and connected to a pressure transducer. In 11 CAL rats and 9 control rats, tracheal resistance was determined immediately after animal death (13). The trachea was exposed and cannulated with a catheter at the level of the main carina to provide a continuous flow (Aalborg Instruments) of compressed air. Tracheal pressure was measured proximal to the circumferential tracheal band. Tracheal resistance was determined as the slope of flow-pressure curves in the linear portion of the curve (0.1–0.5 l/min). In a subset of five CAL and five control animals, arterial blood gases (pH, PCO2, PO2, and HCO3) were determined 14 days after surgery (12, 13, 44).

Fig. 1. Flow chart of ritanserin study protocol. CAL, chronic resistive airway loading.

Table 1. Respiratory changes 14 days after surgery

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Airway Loading</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO2, Torr</td>
<td>90.5 (12.0)</td>
<td>91.1 (11.6)</td>
<td>0.92</td>
</tr>
<tr>
<td>Pco2, Torr</td>
<td>38.0 (4.8)</td>
<td>42.9 (3.1)</td>
<td>0.016</td>
</tr>
<tr>
<td>pH</td>
<td>7.37 (0.05)</td>
<td>7.36 (0.04)</td>
<td>0.85</td>
</tr>
<tr>
<td>Hco3, meq/l</td>
<td>22.3 (3.4)</td>
<td>25.1 (1.7)</td>
<td>0.04</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>11.1 (0.7)</td>
<td>11.5 (0.7)</td>
<td>0.12</td>
</tr>
<tr>
<td>Pes, cmH2O</td>
<td>-5.1 (1.1)</td>
<td>-15.0 (7.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Respiratory rate, breaths/min</td>
<td>96.0 (20.2)</td>
<td>68.8 (23.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tracheal resistance, Torr-l-1-min</td>
<td>6.0 (1.8)</td>
<td>10.0 (4.1)</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Values are means (SD). PO2, arterial O2 pressure; Pco2, arterial CO2 pressure; pH, arterial pH; HCO3, calculated arterial bicarbonate; ΔPes, inspiratory swings in esophageal pressure.
appropriate morphometric software (Media Cybernetics, Silver Spring, MD).

Daily food intake expressed as grams of food per kilogram of body weight was assessed on postsurgery day 12 (44). Animals were housed individually in cages and given 30 g/day (>40% of maximal daily food intake) of standard rodent chow (Harlan, Jerusalem, Israel). The water bottle for each cage was kept full. Food placed in the feeder at the beginning and any left over at the end of each 24-h period were weighed. Any visible food in the cage was scavenged and included in the measurements.

**Ritanserin study.** To examine the effect of ritanserin (2 mg/kg) on growth, circulating GH/IGF-I levels and EGP IGF-I gene expression were determined. Four groups were used: 1) CAL + ritanserin (n = 11), 2) CAL + vehicle (n = 10), 3) control + ritanserin (n = 9), and 4) control + vehicle (n = 8). The vehicle included 2% methyl alcohol in saline. Animals were injected intraperitoneally with 0.6 ml daily immediately after lights on at 0900 for 6 consecutive days, starting 1 day after surgery. Food intake was measured on postsurgery day 5, and somatic growth, serum, tissue (EGP, liver), and tracheal resistance data were collected on day 6, 2 h after the last injection of ritanserin or vehicle (Fig. 1).

Immediately after animal death the serum was separated and frozen for later measurements of GH and IGF-I with specific ELISA kits (DSL, Webster, TX). The liver and tibial EGP cartilage were removed for IGF-I mRNA determination, and the other hind leg was used for EGP morphometry.

**EGP morphometry analysis.** The tibias were processed for paraffin embedding (11). Paraffin sections (5 μm) were deparaffinized in xylene, hydrated in graduated ethanol, and pretreated with 3% acetic acid for 3 min. They were then stained with 1% Alcian blue at pH 2.5 for 30 min, thoroughly rinsed with tap water, and counterstained with hematoxylin and eosin. The size of the EGP was measured by drawing a straight line from the apical border of the reserve zone cells to the lower border of the mineralized cartilage. The findings presented represent the average of 10 measurements in 2 sections from each animal. Morphometric analyses were performed with an Olympus DP-10 digital camera with appropriate morphometric software (Olympus DP-soft, Olympus Optical, New Hyde Park, NY).

**Assays of mRNA and protein levels.** Total RNA was extracted from liver and EGP with the PerfectPure RNA Tissue kit (Gentra Systems, Minneapolis, MN) and used for cDNA synthesis by High-Capacity cDNA synthesis reaction (Applied Biosystems, Foster City, CA). The cDNA samples were then subjected to PCR analysis. Quantitative real-time PCR (qPCR) assays were carried out for IGF-I and β-actin with the following primers: IGF-I sense: GCC TGC TTT TGT AGG CTG CAG TGG, IGF-I antisense: GGA CCA GAG ACC CTT TGC GGG; β-actin sense: CCC GCG AGT ACA ACC TTC T, β-actin antisense: CGT CAT CTA CCG CTA ACT.

qPCR with SYBR Green dye as the detection agent was performed with the ABI Prism 7300 Sequence Detection System (Applied Biosystems, Foster City, CA). The target gene (IGF-I) was normalized for the internal control gene (β-actin) and expressed as transcript-to-housekeeping gene ratio. Each sample was analyzed in triplicate in individual assays. The control group mean was given a value of 1, and individual values are expressed relative to this value.

Reverse transcription-polymerase chain reaction (RT-PCR) and Western immunoblot for protein analysis were performed as described previously (22).

**Data analysis.** Significance between these variables was analyzed by t-test for independent groups. To determine whether tracheal resistance was a predictor of tail and/or tibial length gain, and whether nocturnal Tb was a predictor for MA as the outcome variable, linear regression analysis was performed. One-way analysis of variance (ANOVA-1) was performed for multiple comparisons between groups. The difference between control and CAL groups in Tb, and MA on days 9–13 was analyzed by two-way ANOVA (ANOVA-2) followed by post hoc Newman-Keuls analysis for multiple comparisons. Data are expressed as means ± SD; the null hypotheses were rejected at the 5% level.

<table>
<thead>
<tr>
<th>Table 2. Somatic growth parameters</th>
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<tbody>
<tr>
<td><strong>Control</strong></td>
</tr>
<tr>
<td><strong>Day 0</strong></td>
</tr>
<tr>
<td><strong>Body weight, g</strong></td>
</tr>
<tr>
<td><strong>Tail length, mm</strong></td>
</tr>
<tr>
<td><strong>Tibial length, mm</strong></td>
</tr>
<tr>
<td><strong>Tibial EGP width, μm</strong></td>
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</tbody>
</table>

Values are means (SD). Baseline measurements were made immediately before chronic resistive airway loading (CAL) surgery. Body weight and tail and tibial length gain were measured in 15 control and 15 CAL animals. Histological measurements of epiphyseal growth plate (EGP) were performed on 9 animals for each group; 10 measurements were made on each EGP section, and mean values were compared between groups. *P = 0.002, control vs. CAL on postsurgery day 14; †P < 0.01, day 0 vs. postsurgery day 14; ‡P < 0.0001, control gain vs. CAL gain.

Fig. 3. Correlation of tail (left) and tibial (right) length gains with tracheal resistance. Tracheal resistance measured 14 days after surgery was a significant predictor of both tail and tibial length gains. ◆, Sham-operated control animals (n = 9); ■, obstructed rats (n = 11).
RESULTS

During the procedure the mortality rate of the CAL group was <5%. A total of 80 animals were included in this study: 38 control and 42 CAL animals.

As expected, immediately after CAL, inspiratory swings in esophageal pressure increased by 300%, respiratory rate decreased by 40%, and tracheal resistance increased by 40% (Table 1). Thus the measured changes in inspiratory swings in esophageal pressure and respiratory rate indicate that resistive loading had been produced. CAL animals all demonstrated audible wheezing, especially after activity, but no signs of gasping were observed. Arterial $P_{CO_2}$ and $HCO_3^-$ were significantly greater in CAL animals, with no change in pH (Table 1). Hemoglobin was unchanged in both groups. Daily food intake was 138.7 ± 15.3 and 137.8 ± 16.4 g food/kg body wt in control and CAL animals, respectively ($P = 0.88$).

Continuous recording of $T_b$ and MA for five consecutive days starting from postsurgery day 9 is presented in Fig. 2. Mean $T_b$ of the CAL group was 0.5°C lower compared with the control group ($P < 0.01$, ANOVA-2). Mean light period MA was 2.0 and 1.9 counts/min in CAL and control animals, respectively ($P = 0.7$, ANOVA-2). Mean dark period MA was 5.3 and 6.9 counts/min for CAL and control animals, respectively ($P < 0.01$, ANOVA-2).

Both CAL and control groups had similar baseline body weight and tibial and tail lengths ($P = 0.2$, $P = 0.9$, and $P = 0.9$, respectively). Fourteen days after surgery both CAL and control groups exhibited significant elevation in all growth measurements (Table 2). Growth gain was ~40% less ($P = 0.001$) in CAL animals compared with control animals after the 14-day observation period. Both tail and tibial length gain correlated with tracheal resistance ($r = 0.58$, $P = 0.008$ and $r = 0.57$, $P = 0.01$, respectively) (Fig. 3).

Serum GH levels showed a 38% difference between CAL and sham-operated control animals (124.1 ± 69.2 ng/ml vs. 200.6 ± 91.8 ng/ml; $P = 0.044$). Serum IGF-I levels showed a 32% difference between CAL animals and sham-operated control animals (938.5 ± 172 ng/ml vs. 1,385.5 ± 205 ng/ml in CAL; $P < 0.00002$).

Table 3. Effect of ritanserin on somatic growth parameters and daily food intake

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>C-Rit</th>
<th>CAL</th>
<th>CAL-Rit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 6</td>
<td>Day 0</td>
<td>Day 6</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>53 (8.7)</td>
<td>92 (13.3)*</td>
<td>54 (6.8)</td>
<td>87 (12.2)*</td>
</tr>
<tr>
<td>Tail length, mm</td>
<td>100 (7.0)</td>
<td>128 (5.9)*</td>
<td>97.9 (6.9)</td>
<td>125 (4.6)*</td>
</tr>
<tr>
<td>Tibial length, mm</td>
<td>30.6 (1.6)</td>
<td>36.1 (1.4)*</td>
<td>30.5 (1.1)</td>
<td>35.9 (0.7)*</td>
</tr>
<tr>
<td>Food intake, g/kg body wt</td>
<td>165.9 (11.4)</td>
<td>155.1 (18.1)</td>
<td>170.4 (15.2)</td>
<td>159.3 (8.2)</td>
</tr>
</tbody>
</table>

Values are means (SD). C, control + vehicle; C-Rit, control + ritanserin; CAL, chronic airway loading + vehicle; CAL-Rit, chronic airway loading + ritanserin. Body weight and tail and tibial length were measured on day 0 and day 6. Animals were treated with ritanserin (2 mg/kg) or vehicle for 5 consecutive days. *$P < 0.01$, day 0 vs. postsurgery day 6.
The total width of the EGP cartilage was 31.1% narrower (Table 2) in CAL animals compared with control animals (Fig. 4A). Steady-state EGP IGF-I mRNA levels by RT-PCR assay were measured from pooled EGP homogenates. To obtain a detectable sample it was necessary to pool EGP samples from six animals from each group and assay this as one sample (35). IGF-I mRNA decreased in CAL animals compared with control animals (Fig. 4B). Quantification of the gel with a densitometer revealed an average 38% decrease in IGF-I transcript in CAL animals.

In the ritanserin study, all four groups (i.e., CAL + ritanserin, CAL + vehicle, control + ritanserin, control + vehicle) had similar baseline body weight and tail and tibial lengths ($P = 0.42, P = 0.63, P = 0.54$, respectively). Ritanserin did not affect daily food intake (Table 3), and tracheal resistance was similar in CAL + vehicle and CAL + ritanserin groups: 10.0 (4.1) Torr·l$^{-1}$·min vs. 8.9 (1.9) Torr·l$^{-1}$·min, respectively ($P = 0.46$). There were no significant differences in baseline somatic growth values between all four groups (Table 3). Six days after surgery both CAL and control groups exhibited significant elevation in all growth measurements (Table 3). All growth gain parameters were $\sim$45% less ($P < 0.001$) in CAL compared with control animals after the 6-day observation period. Ritanserin administration for 6 days partially normalized this somatic growth retardation as revealed in tail and tibial lengths, but not in body weight gain (Table 3 and Fig. 5).

Serum GH levels were significantly greater in the CAL + ritanserin group compared with the CAL + vehicle group: 106.9 (25.7) ng/ml and 39.0 (9.9) ng/ml, respectively ($P = 0.031$). CAL decreased serum IGF-I and liver and EGP IGF-I mRNA levels in rats treated with placebo. Ritanserin, however, normalized these serum and organ IGF-I values (Figs. 6 and 7).

**DISCUSSION**

In the present study, upper airway obstruction caused a significant growth delay that was associated with a decrease in the global GH/IGF-I axis and local changes in EGP morphometry and IGF-I gene expression. Pharmacological stimulation of GH release by ritanserin restored both global and local GH/IGF-I levels; however, growth parameters were only partially restored. The following discussion considers these findings and other factors contributing to the delay in growth in the light of limitations of the experimental model and current literature.

This CAL rat model was used previously to investigate adaptive changes in respiratory system function (12, 13, 29, 32, 35, 37, 42–44). Similar to previous studies, we did not find evidence for hypoxia or decline in dietary intake (12, 13, 37, 44). After surgery, inspiratory swings in esophageal pressure and tracheal resistance increased and respiratory rate decreased, similar to what is seen in the adult CAL rat model. Thus resistive loading had been produced. Similar to earlier studies, CAL led to an increase in arterial PCO$_2$ and decreased respiratory rate, suggesting reduction of minute ventilation (13).

Somatic growth was assessed in this study by measuring tibial and tail length gains, which are good indicators of linear growth (11, 39, 43, 44, 47). The process of endochondral ossification, which occurs at the growth plates of the long bones, results in longitudinal growth. This process must be tightly controlled in order to maintain normal growth. The IGF system has been proposed to be the major determinant of postnatal longitudinal growth (36, 40). The IGFs are produced by multiple tissues and can act in both an endocrine and an autocrine/paracrine fashion close to their sites of synthesis.
In our study, circulating GH and IGF-I and local liver and EGP IGF-I production and width were all significantly reduced in CAL animals, suggesting that the growth retardation is compatible with both global and local reduction of IGF-I. Global reduction of GH level will result in reduction of serum, liver, and EGP IGF-I content (10). In addition, a decline in IGF-I expression in the EGP leads to a decrease in its width and contributes to growth retardation (11, 40).

It has long been recognized that sleep is essential for regulation of $T_b$. Partial sleep deprivation leads to hypothermia due to regulation of $T_b$ at a higher level and difficulty in retaining body heat despite increased food intake (9, 33, 34). This decline of $T_b$ can most likely be attributed to impaired sleep and possibly partial sleep deprivation resulting in an inability to maintain $T_b$ and MA (Fig. 2). It is possible that the apparent elevation of respiratory effort following CAL surgery has led to a lighter sleep pattern, necessary to maintain ventilation (1, 15). In children with obstructive sleep apnea, the reduction of serum IGF-I (2, 48) was postulated to be related to shortening of slow-wave sleep duration (2, 25, 45). In rats, circulating GH and IGF-I levels have been observed to diminish compared with prefragmentation baseline periods (8).

Our CAL animals had lower $T_b$ despite preserved (in juvenile rats) (40) or elevated (in adult rats) energy consumption (44). The elevated energy consumption in adult CAL rats was similar to that in previously reported adult rat models of experimental sleep fragmentation (9, 33, 34). In adult humans with obstructive sleep apnea, fragmented sleep leads to increased ghrelin secretion, which results in increased food intake and obesity (30). To our knowledge, little is known on the effect of sleep fragmentation on ghrelin secretion in children with sleep-disordered breathing. Further studies should explore ghrelin levels in juvenile and adult CAL models.

Pharmacological stimulation of GH release by ritanserin restored both global and local GH/IGF-I levels; however, growth parameters were only partially restored. The lack of normalization in growth parameters following ritanserin treatment could suggest that other mechanisms are involved, such as impairment of total body energy balance due to increased work of breathing. Previously we demonstrated (13, 44) that total body energy balance (oxygen consumption/daily food intake), hemoglobin and lactate levels, and arterial oxygenation were similar to those of the control group, within the normal physiological range during quiet wakefulness. However, when severe upper airway loading was induced, adult rats exhibited a considerable decrease in body weight immediately after surgery that was related to reduction of food and water intake and severe hypoxemia (29). Strenuous muscle contraction and hypoxemia are known to induce protein loss and cachexia (35). Similar to previous studies, we did not find evidence for hypoxia (12, 13, 37, 44). Hypoxia can serve as an additional potential trigger of reduced dietary intake (7, 16, 38) and decreased circulating IGF-I and IGF-binding protein (IGFBP)-3 (4, 18, 26, 27). Further studies are needed to determine whether CAL induces oxidative stress and activation of proinflammatory processes in the respiratory muscles.

Pharmacological stimulation of GH release by ritanserin restored both global and local GH/IGF-I levels. Gronfier et al. (14) reported a considerable increase in delta wave sleep activity that was paralleled by a similar increase in GH secretion rate after sleep onset in humans administered ritanserin versus placebo control subjects. Augmented GH release following administration of pharmacological agents may represent a novel class of GH secretagogues (14, 46). It is possible that the improved longitudinal growth following ritanserin administration in the present study was related to enhancement of slow-wave sleep (20) and its sleep-consolidating effect (21).
Our study has several limitations that should be recognized. In the present study we introduced both inspiratory and expiratory upper airway loading, while in clinical sleep-disordered breathing upper airway loading is mainly inspiratory and sleep related (17, 24). The respiratory changes following CAL are not solely related to sleep. The increased swings in esophageal pressure in the absence of significant changes in arterial PO2 (gas exchange abnormalities) seen in our animals may be relevant for a variety of respiratory disorders in children, including tracheomalacia, tracheal stenosis, and upper airway resistance syndrome (1, 15). Increased tracheal resistance after surgery was found to be a significant predictor of both tail and tibial length gain failure. However, tracheal resistance was quantified after animal death without any muscle activity. In animals and humans, upper airway resistance changes considerably during the sleep-wake cycle (17, 24, 31). Upper airway resistance in our rats was determined by CAL surgery and alterations in central ventilatory drive due to the loading response (12, 13). Thus the apparent correlation of tracheal resistance with growth should be further explored in intact animals during sleep and wakefulness. GH secretion occurs in pulses throughout the day, but deep slow-wave sleep shortly after sleep onset is associated with particularly large bursts of GH secretion from the anterior pituitary somatotrophic cells (8). In the present study we used MA and Tb as surrogates of GH secretion from the anterior pituitary somatotrophic cells after sleep onset is associated with particularly large bursts of GH secretion from the anterior pituitary somatotrophic cells (8). In the present study we used MA and Tb as surrogates of GH secretion from the anterior pituitary somatotrophic cells after sleep onset.

REFERENCES


