Upper airway extraluminal tissue pressure fluctuations during breathing in rabbits

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The major pathophysiological feature of the obstructive sleep apnea (OSA) syndrome is collapse of the pharyngeal airway during sleep. Over the last two decades, recognition of this sleep-related abnormality in upper airway patency control has generated considerable interest in determining factors that influence upper airway mechanical properties during sleep. The "balance of forces model," first proposed by Remmers et al. in 1978 (12), views the patency and stability of the upper airway as being dependent on the action of upper airway dilator muscles that normally demonstrate rhythmic activation during inspiration. Upper airway collapse occurs when the dilating forces produced by these muscles are exceeded by the negative airway pressure generated by the respiratory muscles during inspiration. Sleep-related decrements in upper airway muscle activity, when combined with an anatomically small-diameter upper airway lumen, have been postulated as a principal pathogenic pathway for episodic upper airway narrowing and collapse during sleep (8). In the 1990’s, Isono and Remmers (3) modified the “forces” model and introduced the “balance of pressures” model, which stressed the pivotal role of upper airway wall transmural pressure (defined as “the difference between intraluminal and tissue pressure”). This model introduced the concept that tissues surrounding the upper airway exert a mechanical pressure on the airway wall. In this analysis, upper airway extraluminal tissue pressure (ETP) is expressed as the vector sum of the mechanical pressures exerted on upper airway walls by surrounding tissue structures. Because airway intraluminal pressure varies with phase of respiration and ETP may not be a constant, the resulting transmural pressure may be compressive or decompressive with respect to the upper airway lumen.

The pivotal influence of upper airway ETP as a unifying mechanism linking surrounding tissue structures to upper airway patency is a recurring feature of a number of contemporary analyses of upper airway mechanics. These include linkage of ETP to critical closing pressures in the upper airway via the Starling resistor model (18), demonstration of pharyngeal occlusion during central apnea (2), the model of tracheal traction by Rowley and colleagues (13), and studies linking ETP with neck circumference and upper airway compliance during sleep (14). In addition, Isono and colleagues (6) recently used an ETP-based paradigm to explain the posture dependence of pharyngeal wall compliance.
Whereas there is considerable theoretical and indirect evidence supporting the ETP model (2, 3, 6, 13, 14, 18), there are remarkably few experimental data. No direct data describe upper airway ETP in human subjects, although some measurements have been made in animals, particularly with regard to pressures operating within the lateral fat pads surrounding the pharynx (23, 24). However, there have been no further studies aimed at defining the determinants of local ETP values or at unraveling the functional interactions between ETP and upper airway patency.

Using the methodology first published by Winter et al. (23, 24), we have developed an anesthetized rabbit model in which local upper airway ETP can be measured and functional relationships with upper airway patency explored. In the present report, we describe the features of this model, examine ETP at different sites around the pharyngeal airway, and examine the hypothesis that ETP is influenced by alterations in head and neck position, resistive loading, and stimulated breathing.

MATERIALS AND METHODS

Subjects. Studies were performed in 28 adult male New Zealand White rabbits (weight: 2.5–3.5 kg). The protocol was approved by the Western Sydney Area Health Service Animal Ethics Committee.

Anesthesia. Anesthesia was induced with an intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg) and maintained by using intravenous ketamine (15 mg·kg⁻¹·h⁻¹) and xylazine (4.5 mg·kg⁻¹·h⁻¹).

Measurement of ETP. Upper airway ETP was measured in spontaneously breathing supine rabbits by using pressure transducer-tipped catheters (Millar MPC 500, Millar Instruments, Houston, TX) surgically inserted into the tissues surrounding the pharyngeal airway (23, 24). For measurement of later pharyngeal wall ETP (ETPat), a skin incision was made at the level of the angle of the mandible on the right side. Blunt dissection of subcutaneous tissues was used to expose the pharyngeal wall submucosa. The transducer catheter tip, with its sensor oriented toward the pharyngeal lumen, was positioned within the submucosal tissues (i.e., immediately adjacent to the pharyngeal wall mucosa) and then sutured in place by using a purse string suture (5.0 prolene, Davis and Geck). The skin incision was then closed (0 silk, Davis and Geck). For studies in which anterior pharyngeal wall ETP was also measured (ETPant; n = 20), a similar procedure was performed to insert a second transducer-tipped catheter into the tissues of the anterior pharyngeal wall midline in the coronal plane and level with the angle of the mandible. Correct positioning of each catheter in the submucosa between the oropharynx and epiglottis was verified via postmortem dissection at the conclusion of each study.

Experimental setup. A conical small-animal anesthetic mask (no. 2, GaleMed) with attached pneumotachograph (Fleisch model no. 00, Harvard Apparatus) and pressure transducer (±5.6 cmH₂O, Validyne) was then fitted tightly over the rabbit’s snout to allow monitoring of mean pressure (Pm) and airflow (see Fig. 1). The airflow signal was integrated on-line to obtain tidal volume (VT). Rabbits were secured in a specially designed apparatus that allowed flexion of the head in the sagittal plane but prevented movements in any other plane. Data were digitized (MacLab 16s, ADInstruments, Sydney, Australia) and stored on a Macintosh computer for later analysis.

Preliminary methodological studies. Preliminary studies were conducted in two additional rabbits to test the influence of transducer sensor orientation on ETP values and to evaluate baseline stability. In the first rabbit, a transducer-tipped catheter was inserted into the lateral wall, as described above (i.e., with the transducer oriented toward the pharyngeal wall). Head position was then changed for a total of nine times between 30 and 70°. The catheter was then removed and reinserted with the transducer sensor oriented toward the skin (i.e., away from the pharyngeal wall), and the series of head position changes was repeated. In the second rabbit, a catheter was inserted into the anterior pharyngeal wall with the head and neck at 30° to the horizontal (see Fig. 1), and the signal was recorded continuously for 1 h with no interventions being performed.

Head and neck flexion. During resting tidal breathing, measurements of ETP (three runs of 5–10 steady-state breaths) were obtained with head and neck positions of 30, 50, and 70° (measured as the angle between the horizontal and a line drawn from the tragus to the external nares; see Fig. 1).

Resistive breathing and occlusion. During resistive loading, rabbits breathed via the face mask with an attached external resistor (internal diameter, 2 mm) for a period of 2 min (n = 10). After a recovery period, the mask was then completely occluded for a period of 20 s. The effect of the external resistive load (immediately after addition of resistor) and brief mask occlusion was studied at each of the three different head positions.

Hypercapnic hyperventilation. The effect of hypercapnic hyperventilation on ETP was assessed by having rabbits (n = 9) re-
breathe from a rubber anesthetic bag containing 3 liters of 5% carbon dioxide in oxygen attached to the face mask until VT doubled.

Data analysis. For each head and neck position, three runs of 5–10 steady-state breaths were analyzed for individual breath measurements for ETPlat, ETPant, VT, and peak inspiratory Pm. The ETP data for each breath were expressed as a maximum, minimum, and mean (average value for each breath) value, as well as change (Δ) in ETP (i.e., maximum – minimum; ΔETP). Data were then averaged for each run. For each rabbit, run data were then pooled to obtain mean data for each condition. Mean individual rabbit data were then averaged to obtain group mean (±SE) data. The within-individual rabbit (random effect) interactions between head and neck position (fixed effect), transducer position (fixed effect, lateral vs. anterior), and ETP values were analyzed by using a linear mixed-effects model (11). A similar analysis was also used to examine within-individual rabbit (random effect) interactions between head and neck position (fixed effect), transducer position (fixed effect, lateral vs. anterior), VT (covariate), and ETP outcomes during hyperpnea. Transducer orientation, resistive loading, and mask occlusion data were analyzed by using repeated-measures ANOVA, with a Bonferroni post hoc test. P < 0.05 was considered significant.

RESULTS

Preliminary methodological studies. There was no significant effect of transducer orientation on measured ETPlat values at either head and neck position (all P > 0.05). Baseline drift for ETPant was ~0.1 cmH2O/h.

Topographical variation and head and neck flexion. Raw data for one rabbit are shown in Fig. 2. Over the range of conditions studied, within-rabbit baseline ETP values tended to be above atmospheric pressure, fluctuated with phase of respiration, and were often greater for ETPlat than for ETPant. In the majority of rabbits, ETP decreased in phase with inspiration and increased with expiration. Increased head and neck flexion was associated with increased ETP values. VT varied between rabbits (range: 6.3–24.3 ml), but group mean values were not significantly influenced by head and neck position (all P > 0.9). Group mean (±SE) values for ETPant and ETPlat at each head and neck position are shown in Fig. 3. Progressively increasing head and neck flexion resulted in a progressive increase in the maximum, minimum, Δ, and mean ETPlat and ETPant. Mean ETPlat was greater than mean ETPant at all head and neck positions (all P < 0.05; see Fig. 3).

When the data were analyzed by using the linear mixed-effects model, there was no significant interaction (P > 0.05) between site of measurement and degree of head and neck flexion for any ETP outcome measure (i.e., both ETPlat and ETPant responded to changes in head and neck position in the same manner). However, both degree of head and neck flexion and site of measurement were themselves significant independent predictors of ETP values. Mean ETPlat (P < 0.05) was ~0.7 cmH2O greater than mean ETPant when adjusted for degree of head and neck flexion. Mean, maximum, and minimum ETP values increased significantly by 0.7–0.8 cmH2O/20° of head and neck flexion when adjusted for site of measurement (P < 0.0001). ΔETP was not influenced by measurement.
site, but both ΔETPlat and ΔETPant increased by ~0.2 cmH\(_2\)O for every 40° of head and neck flexion (P < 0.005).

Resistive breathing and occlusion. During resistive loading, there was a significant decrease in peak inspiratory PM from -0.3 ± 0.5 to -1.9 ± 0.4 cmH\(_2\)O for head and neck position of 30°, -0.3 ± 0.6 to -2.1 ± 0.2 cmH\(_2\)O (50°), and -0.3 ± 0.1 to -1.7 ± 0.2 cmH\(_2\)O (70°) (all P < 0.05). VT significantly decreased with resistive loading from 13.5 ± 1.3 to 9.9 ± 1.4 ml (30°), 14.3 ± 1.5 to 10.9 ± 1.2 ml (50°), and 13.8 ± 1.1 to 9.7 ± 0.8 ml (70°) (all P < 0.05). Mask occlusion resulted in a further significant fall in peak inspiratory Pms to -4.7 ± 0.6 cmH\(_2\)O (30°), -5.6 ± 0.6 cmH\(_2\)O (50°), and -4.8 ± 0.6 cmH\(_2\)O (70°) (all P < 0.001 compared with baseline). The main effect of resistive loading and occlusion on ETP values was an increase in ΔETPlat and ΔETPant at all head and neck positions (Table 1).

Hypercapnic hyperpnea. During CO\(_2\) breathing, VT increased significantly in all rabbits, reaching 16.6–31.3 ml (P < 0.001 for all head positions). Figure 4 shows the relationships between VT and mean ETP values. When analyzed using the mixed linear effects model, maximum, minimum, Δ, and mean ETPlat and ETPant all increased with increasing VT at the head and neck position of 30° (Table 2, all P < 0.05). However, this relationship was modified by head and neck position, such that, at 50 and 70°, there was no significant effect of VT on ETPlat values (all P > 0.05). At 50°, maximum, minimum, Δ, and mean ETPant increased with increasing VT, and at 70° the relationship between VT and ETPant values was reversed (all P < 0.05, Table 2).

Table 1. Effect of resistive loading or brief mask occlusion on mean, maximum, minimum, and ΔETP in lateral and anterior pharyngeal wall

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<td>TB</td>
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<td>OCC</td>
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<tr>
<td>Mean ETP</td>
<td>2.7 ± 1.2</td>
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<td>Maximum ETP</td>
<td>2.9 ± 1.2</td>
<td>3.0 ± 1.2</td>
<td>3.2 ± 1.1*</td>
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<td>Minimum ETP</td>
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<td>2.4 ± 1.1</td>
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<td>ΔETP</td>
<td>0.6 ± 0.1</td>
<td>0.7 ± 0.1*</td>
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<tr>
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<td>Maximum ETP</td>
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<td>Minimum ETP</td>
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<td>ΔETP</td>
<td>0.8 ± 0.1</td>
<td>1.0 ± 0.1*</td>
<td>1.2 ± 0.2*</td>
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Values are means ± SE in cmH\(_2\)O; n, no. of animals. TB, tidal breathing (no load); RB, resistive loading; OCC, mask occlusion; ETP, extraluminal tissue pressure; Δ, change. *P < 0.05 compared with tidal breathing at each respective head position, repeated-measures ANOVA.
Fig. 4. Plot showing linear regression line for the relationship between mean ETPlat (solid lines) and mean ETPant (dashed lines) and tidal volume (VT) for each of the 3 head and neck positions, as determined by the linear mixed effects model (n = 9). Changes in VT were obtained by using hyperoxic hypercapnia. Note that, at head and neck position of 30°, increasing VT is associated with an increase in both mean ETPlat and ETPant. Increasing head and neck flexion reduces or abolishes this relationship, until, at head and neck position of 70°, increasing VT is associated with a decrease in mean ETPant. *P < 0.05 for slope.

**DISCUSSION**

This study has demonstrated that, in spontaneously breathing anesthetized rabbits, upper airway ETP is usually positive and fluctuates with respiration, increasing with expiration and decreasing with inspiration. These findings are qualitatively similar to previously published data from upper airway lateral fat pads in pigs (23, 24). However, we have now demonstrated for the first time that ETP is nonhomogeneously distributed around the upper airway, with ETP values in supine rabbits being consistently greater for the lateral pharyngeal wall than for the anterior pharyngeal wall. Furthermore, upper airway ETP values increase with both increasing head and neck flexion and increasing VT. External resistive loading and brief airway occlusion tend to increase the size of respiratory-related fluctuations in upper airway ETP.

**Critique of methods.** We utilized the methodology developed by Winter and colleagues (23, 24) for our studies in anesthetized rabbits. While the limitations of this approach have been described previously (23, 24), we will briefly examine some of the potential sources of error. The introduction of the catheter probably results in an alteration in the absolute pressure present in the tissues, because it acts as a space-occupying mass. Thus our measurements of ETP in absolute terms may not accurately represent those present when the system has not been perturbed by the introduction and presence of the catheter. However, most of our data analyses rely on a comparison of a postintervention measurement with a baseline value. Thus we predominantly deal with change in ETP values rather than absolute levels. During our preliminary methodological studies, we demonstrated that the orientation of the transducer tip of the catheter did not influence the measurement and that the signal did not drift substantially. Consequently, baseline values were usually stable, allowing the effect of interventions to be clearly demonstrated (see Fig. 2). The transducer is placed in contact with the submucosa of the pharyngeal wall. Thus we measure the mechanical pressure that exists immediately adjacent to the air-tissue interface. This pressure is representative of the local vector sum of all of the pressure-generating influences acting on the airway wall at the point of measurement. Thus we believe that the results obtained represent a consistent reflection of the local pressure in the tissues surrounding the upper airway wall.

The ETP values measured in the present study were largely positive, a finding that suggests that, predominantly, ETP exerts a collapsing force on the upper airway wall. This finding is consistent with theoretical analyses presented by a number of previous authors. During sleep, the human pharyngeal airway behaves as a Starling resistor (18), suggesting that pharyngeal closure will be related to the difference between intraluminal pressure and surrounding ETP. In addition, in sleeping patients with OSA, upper airway narrowing is not confined to inspiration (i.e., when intraluminal pressures are negative), but can also occur during expiration (i.e., when intraluminal pressures are positive) (9, 15). Pharyngeal airway narrowing has also been demonstrated in sleeping human subjects during central apneic episodes (2), indicating that negative intraluminal pressures are not a necessary condition for upper airway narrowing or collapse. Under general anesthesia, with a passive upper airway, closing pressures in patients with OSA are frequently positive, a finding that also suggests a collapsing force exerted by surrounding ETP (5). All of these

| Table 2. Slopes of the relationships between tidal volume and ETPlat and ETPant at each of the 3 head positions obtained by using the linear mixed-effects model analysis |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | 30°  | 50°  | 70°  | 30°  | 50°  | 70°  |
| Mean ETP       | 0.07 ± 0.01 | NS   | NS   | 0.07 ± 0.01 | 0.03 ± 0.01 | −0.06 ± 0.01 |
| Maximum ETP    | 0.08 ± 0.01 | NS   | NS   | 0.07 ± 0.01 | 0.04 ± 0.01 | −0.06 ± 0.01 |
| Minimum ETP    | 0.07 ± 0.01 | NS   | NS   | 0.07 ± 0.01 | 0.02 ± 0.01 | −0.06 ± 0.01 |
| ΔETP NS (P = 0.06) | NS   | NS   | NS   |

Values are means ± SE in cmH2O/ml. ETPlat, lateral pharyngeal wall ETP; ETPant, anterior pharyngeal wall ETP; NS, no significant slope. All data P < 0.05.

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reports support the notion that upper airway ETP is likely to be greater than atmospheric pressure.

We also found that, in supine rabbits, ETP was nonuniformly distributed around the upper airway, with values measured around the superior and lateral pharyngeal wall being greater than those around the inferior and anterior pharyngeal wall. This implies that, at any level within the upper airway, transmural pressure may not be circumferentially uniform. This raises the potential for upper airway cross-sectional area and shape to be, at least partially, determined by the distribution of extraluminal tissue pressures acting across the airway wall. Different ETP values for the lateral and anterior walls may be related to different local mechanical linkages between surrounding tissue structures and the airway wall mucosa and/or the influence of gravity, given that, in the supine rabbit, the ETPlat site is superior to the ETPant site.

In supine human subjects, the pharyngeal airway is nonuniform in shape, with narrowing of the upper airway walls laterally, particularly in subjects with OSA (16, 20). Lateral narrowing in subjects with OSA is related to the thickness of the lateral pharyngeal walls (17), a phenomenon that may be reflective of, or may itself lead to, an increased lateral ETP. If our findings in supine rabbits are representative of ETP distributions in human subjects, then lateral wall narrowing in humans may be related to a nonuniform ETP distribution.

Gravitational effects on ETP values have been invoked recently by Isono and colleagues (6), who demonstrated that the size of the pharyngeal airway in anesthetized humans is greatly affected by body position, with an increase in upper airway cross-sectional area (at the same level in the upper airway) in the lateral position compared with supine. These authors suggest that the tissue mass overlaying the pharyngeal airway in supine humans is greater than in the lateral posture. They speculate that a greater gravitationally mediated ETP results in a greater compressive transmural pressure in the supine vs. the lateral posture. Thus we speculate that one possible cause of regional differences in ETP may be the effect of gravity on the tissue mass surrounding any level within the pharyngeal airway.

Head and neck flexion. Compression of the upper airway extraluminal tissue space with increasing head and neck flexion resulted in an increase in both ETPlat and ETPant. Flexion of the head and neck has been shown in animal models to increase the collapsibility of the upper airway (10, 21), and, in human subjects, head flexion increases upper airway resistance (1), decreases upper airway size (7), and increases upper airway collapsibility (4). We now suggest that these reported effects of head and neck flexion on upper airway patency may be mediated via changes in local upper airway ETP. Moreover, our findings provide some insight into the complex mechanical interactions that contribute to the determination of local upper airway ETP and subsequent segmental upper airway wall transmural pressure.

Resistive breathing and occlusion. The addition of an external resistor or an occlusion resulted in no change in the mean ETPlat or ETPant; however, both \( \Delta \text{ETP} \) increased slightly with mask occlusion. Fluctuations in ETP were in phase with respiration, increasing with expiration and decreasing with inspiration. These fluctuations were also observed in pigs by Winter and colleagues (23, 24), who speculated that they were due to tracheal traction on the upper airway, upper airway muscle contraction, or transmitted pressures from the upper airway lumen. In dogs, Van de Graaf (22) demonstrated that, during tracheal occlusion, the resistance of the isolated upper airway decreases and the trachea moves caudally. This finding suggests that the increase in respiratory-related ETP fluctuations seen in the present study with airway occlusion may still be due to increased tracheal traction or, as suggested by Van de Graaf, transmission of pleural pressures into the neck. Similarly, the increase in ETP fluctuation seen with head and neck flexion may be due to similar mechanisms associated with an increase in upper airway resistance (1).

Hypercapnic hyperpnea. Increasing \( V_t \) with hyperoxic hypercapnia was associated with an increase in maximum, minimum, \( \Delta \), mean ETPlat, and ETPant; however, this relationship was lost with increasing head and neck flexion. \( \Delta \text{ETP} \) may be reflective of increased tracheal traction forces associated with an increased \( V_t \) (potentially leading to a relatively lower peak inspiratory ETP value), whereas the increase in mean ETP with increasing \( V_t \) may be related to an increase in upper airway dilator muscle activation (19) associated with hypercapnia. However, this relationship between \( V_t \) and ETP is overridden by the more powerful compression for the upper airway extraluminal tissue space associated with increasing head and neck flexion.

We conclude that the pressure in the tissues surrounding the pharyngeal airway is usually positive and thus exerts a collapsing pressure on the upper airway. This pressure, however, is nonhomogeneously distributed around the pharyngeal airway wall and is increased with increasing \( V_t \) and head and neck flexion. We speculate that upper airway ETP is influenced by gravity, transmitted pressures from the upper airway lumen and possibly the pleural space, upper airway muscle activity, and mechanical linkages, such as head and neck position and lung volume-related caudal tracheal traction. The pressure exerted on pharyngeal airway walls by surrounding tissue structures appears to be determined by a complex interaction of local mechanical interdependencies that are influenced by both postural and respiratory-related factors.

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DISCLOSURES

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