Mass loading of the upper airway extraluminal tissue space in rabbits: effects on tissue pressure and pharyngeal airway lumen geometry

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Lateral pharyngeal fat pad compression of the upper airway (UA) wall is thought to influence UA size in patients with obstructive sleep apnea. We examined interactions between acute mass/volume loading of the UA extra-luminal tissue space and UA patency. We studied 12 supine, anesthetized, spontaneously breathing, head position-controlled (50°), New Zealand White rabbits. Submucosal extraluminal tissue pressures (ETP) in the anterolateral (ETPlat) and anterior (ETPant) pharyngeal wall were monitored with surgically inserted pressure transducer-tipped catheters (Millar). Tracheal pressure (Ptr) and airflow (V˙) were measured via a pneumotachograph and pressure transducer inserted in series into the intact trachea, with hypopharyngeal lateral pharyngeal fat pad compression of the upper airway (18, 21). In this analysis, the functional determinant of reduced upper airway lumen size is the increased upper airway extraluminal tissue pressure (ETP) that is hypothesized to occur with relatively large tissue volumes and/or relatively small enclosure volumes (18).

There are no reported measurements of upper airway ETP measurements in human subjects, but insights have been gained using animal models. The first reported tissue pressure measurements were in the lateral peripharyngeal fat pads of pigs (22, 23). More recently, our laboratory has developed an anaesthetized supine rabbit model in which it was demonstrated that peripharyngeal tissue pressure is nonuniformly distributed around the upper airway, being greater in the lateral (EPTlat) than the anterior (EPTant) tissues (5, 6). Studies of human subjects with OSA have demonstrated altered distribution of the peripharyngeal tissues when compared with healthy subjects. In particular, OSA patients have increased tongue and lateral pharyngeal wall size (13, 15). Indeed, even nonobese subjects with OSA have excess fat laterally to their airway and increased neck tissue volume (9, 16, 19). These findings suggest that both the volume and topography of the peripharyngeal tissue mass exert an influence on pharyngeal airway lumen geometry.

In support of this concept, previous animal studies have demonstrated a reduction in the cross-sectional area of the retropalatal airway, and an increase in upper airway resistance (R ua), when local peripharyngeal tissue volume was increased acutely by inflation of balloons implanted in the tissues of the lateral pharyngeal wall (21). However, in these studies, upper airway extraluminal tissue pressure was not measured.

Upper airway patency is determined by the transmural pressure, or the difference between the intraluminal pressures and extraluminal tissue pressure (11). We hypothesize that peripharyngeal tissue volume may exert an effect on pharyngeal lumen geometry such that an acute local increase in peripharyngeal tissue volume will result in a change in the distribution of peripharyngeal extraluminal tissue pressure accompanied by local narrowing of the pharyngeal airway lumen. It is possible that, in response to acute increases in the tissue volume, there may be movement of the tissues outside of the restraints of the surrounding bony enclosure (18) and no resulting increase in tissue pressure. Consequently, to test of the validity of our hypothesis, we implanted, and then acutely inflated, silicone tissue expander balloons in the peripharyngeal tissues of anesthetized, supine, rabbits.
METHODS

Subjects. Studies were performed on 12 adult, male, anesthetized (ketamine and xylazine), supine, spontaneously breathing, New Zealand White rabbits. Animals were studied with head and neck position controlled such that a line drawn from the tragus to the external nares was at 50° to the horizontal (5). The protocol was approved by the Western Sydney Area Health Service Animal Ethics Committee.

Measurement of upper airway ETP. The upper airway ETP was measured using pressure transducer tipped catheters (Millar MPC 500, Millar Instruments, Houston, TX) surgically inserted into the tissues surrounding the pharyngeal airway as previously described (4–6). Briefly, pressure was measured in the pharyngeal submucosal tissues at the level of the angle of the mandible (1) the right anterolateral ETPlat and 2) the ETPant midline in the coronal plane. Correct positioning of each catheter was verified via computed tomography (CT) images (see below) or via postmortem dissection at the conclusion of each study.

Measurement of airflow and pressures. Respiratory airflow (V) was measured using a heated pneumotachograph (Hans-Rudolph 8300A, Hans Rudolph, Kansas, MO) inserted in series between the third and tenth ring of the intact trachea as described previously (6). Driving pressure for V was measured as tracheal lumen pressure (Ptr) using a pressure transducer (±10 cmH2O, Celsco) connected to a side port in the cranial tracheal cannula (see Fig. 1).

Peripharyngeal tissue expanders. A compliant silicone, pediatric, orbital tissue expander prosthetic balloon (maximal volume 3 ml, Silimed Brazil) was surgically implanted subcutaneously above the tissues surrounding the anterolateral pharyngeal wall, immediately medial to the angle of the mandible and directly above the sensor of the anterolateral pressure transducer-tipped catheter. Three runs of graded (0–1.5 ml in 0.5-ml increments) saline inflations of the tissue expander were performed while monitoring ETPlat, ETPant, V, and driving pressure for V (see Fig. 2).

Pharyngeal airway imaging. CT imaging of the upper airway was performed on six of the rabbits during graded saline tissue expander balloon inflations and with head and neck position controlled (50°) and while recording V and driving pressure for V, ETPlat, and ETPant. Images were acquired on an Aquilion 16-slice system (Toshiba, Tokyo, Japan). The volume of interest was identified on a lateral projection scanogram (120 kV, 80 mA), and helical image acquisition was then performed [120 kV, 100 mA, 0.5-s rotation, 16 × 0.5-mm detectors, 5.5 mm/s couch speed, 1:11 helical pitch, direct field of view (180 mm) scan field]. Image reconstruction was performed using a 512 × 512 image matrix, filter convolution 50 reconstruction algorithm, and 0.5-mm slice thickness with 0.4-mm interval (i.e., 0.1-mm overlap).

Data analysis: imaging data. Each image data set was loaded into a multiplanar reformat platform and manipulated so that the long axis of the first cervical vertebra was horizontal. Axial images (1.7–1.9 mm thick) were then generated from the occipitoaxial junction to the glottis. An elliptical-shaped region of interest was placed over each of the axial images of the pharyngeal airway and its surrounding margins, and the cross-sectional area of the pharyngeal lumen was measured by limiting the range of the CT numbers between −200 and −1,536 Houndsfield units; thus only CT numbers equivalent to air were examined and included in the area calculation. Axial image position was expressed as a function of percentage of airway length as measured from the occipitoaxial junction to the glottis. For each rabbit, the average cross-sectional values for each of the three runs were sorted into 10% bins along the airway length.

Data analysis: V and pressure. Mean ETP values (average value) were obtained by analyzing three runs of segments five breaths in duration, which were stable in terms of ETPlat, upper airway pressure, and V for each condition studied. To calculate upper airway resistance (Rua), power functions [Ptr = aVb + c, where a, b, and c are constants, where the exponent b can be used to describe the flow regime (20)] were fitted to the inspiratory limb of pressure vs. V plots generated from each run of five breaths for each load over a respiratory V range of 0–20 ml/s (6). Rua (Ptr/V at V = 20 ml/s) was calculated from the fitted power function and expressed as a percentage of baseline Rua.

Statistical analysis. Individual run data were averaged to obtain individual rabbit data that were then pooled to obtain group mean data. Data were compared using repeated-measures ANOVA at with a Bonferroni post hoc test for comparison with baseline. For the change in cross-sectional areas, a repeated-measures ANOVA was performed.
was performed at each level along the airway. We also examined the changes in ETPlat and ETPant using linear mixed effects modeling, assuming that rabbit identity and tissue expander inflation were random effects and that site of tissue pressure measurement was a fixed effect. \( P < 0.05 \) was considered significant.

**RESULTS**

**ETP.** Technically acceptable measurements of EPTlat and ETPant were obtained in 11 and 9 rabbits, respectively. The major reason for discarding the measurements was because on review of the images it was evident that there was poor catheter placement. Data were discarded for one measurement of anterolateral tissue pressure and three measurements of anterior tissue pressure. In total, there were eight rabbits with complete sets of data. At baseline, mean ETPlat was \( 2.3 \pm 0.5 \text{ cmH}_2\text{O} \), and with expansion of the tissue expander to 1.5 ml the mean ETPlat increased to \( 5.0 \pm 1.1 \text{ cmH}_2\text{O} \) \( (P < 0.05) \); Fig. 3 and Fig. 4). At baseline, mean ETPant was \( 0.5 \pm 0.5 \text{ cmH}_2\text{O} \) with no significant change with tissue expander inflation \( (P = 0.17) \). When examined with the linear mixed effects model, there was a significant interaction between the measurement sites. For every milliliter of tissue expander inflation, ETPlat increased by \( 2.19 \pm 0.6 \text{ cmH}_2\text{O} \) \( (P < 0.001) \); however, there was no significant increase in ETPant \( (P = 0.4) \).

**Rua.** Inflation of the tissue expander to 1.5 ml resulted in a significant increase in inspiratory Rua (at 20 ml/s) to \( 119 \pm 4.2\% \) of the baseline value \( (n = 12; P < 0.001; \text{mean \pm SE}; \text{Fig. 3 and Fig. 4}) \).

**Pharyngeal lumen cross-sectional area.** For the group, inflation of the antero-lateral tissue expander to 1.5 ml resulted in a significant decrease in pharyngeal lumen cross-sectional area over a region situated between 10 and 70% of the total airway length (Fig. 5). The reduction in cross-sectional area was not confined to the position of the tissue expander, but it also...

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**Fig. 2.** Axial computed tomography image from 1 rabbit after before \( (A) \) and after \( (B) \) inflation of the tissue expander to 1.5 ml. The hypopharyngeal airway is indicated, as is the position of the anterolateral and anterior pressure transducer-tipped catheters. The diameter of each of the pressure transducer-tipped catheters is 2 mm.

**Fig. 3.** Raw data from 1 rabbit demonstrating the effect of graded inflation of the anterolateral tissue expander on ETPlat, ETPant,Ptr, and V. Note that with graded inflation of the tissue expander there is a gradual increase in ETPlat, little change in ETPant, an increase in the driving pressure for V, and the development of flow limitation. Inspiration is upward on V trace.

**Fig. 4.** At baseline, mean ETPant was \( 0.5 \pm 0.5 \text{ cmH}_2\text{O} \) with no significant change with tissue expander inflation \( (P = 0.17) \).
DISCUSSION

These findings demonstrate that an acute increase in the volume of the peripharyngeal tissues results in a localized increase in the magnitude of the compressive peripharyngeal tissue pressure exerted on the airway wall. This change in the distribution of upper airway extraluminal tissue pressure is accompanied by a reduction in the pharyngeal lumen cross-sectional area in the hypopharynx and an increase in Rua. These findings are compatible with previous hypotheses (3, 18, 21) that increased volume of peripharyngeal tissues will be linked with reduced local airway lumen size via an increase in the local surrounding peripharyngeal tissue pressure.

Critique of methods. The methodology used for measuring upper airway extraluminal tissue pressure have been critiqued elsewhere (5, 6). However, an important limitation is that the invasive introduction of the pressure transducer-tipped catheter into the tissues is likely to perturb the baseline tissue pressure. Consequently, we have focused on changes in rather than the absolute magnitude of the pressures measured. We have also measured peripharyngeal tissue pressures at only two sites; results may differ if peripharyngeal tissue pressures was measured at other sites.

To increase tissue volume, we used inflatable tissue expanders. These were chosen because they are compliant when inflated. This is similar to the approach taken by Winter et al. (21). The magnitude of the volume loads used in study were based on reported volumes for neck fat volume within the jaw line in human subjects with OSA of 133 ml (9) and then scaled for rabbit body weight. Consequently, the loads used in our study are smaller and in a more physiological range than those Koenig et al. (7) used in rabbits, and they also were less than loads used in pigs (21).

Fig. 4. Individual (A, C, E) and grouped (B, D, F; mean ± SE) showing the effect of graded inflation of the anterolateral tissue expander on inspiratory upper airway resistance (Rua; A and B) and on the change from baseline in the pressures in the lateral (ΔETPlat; C and D) and anterior (ΔETPant; E and F) pharyngeal wall. Individual rabbits are represented by different symbols. *P < 0.05 compared with baseline. Note that there is an increase in pressures in the tissues in the anterolateral pharyngeal wall, associated with a reduction in inspiratory Rua with no change in ETPant.

occurred above the position of the tissue expander in all six rabbits.

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During the study, the rabbit head and neck was positioned at 50° to the horizontal. Our laboratory has previously shown that head and neck position influences peripharyngeal tissue pressure (5); thus our findings may be specific to this particular head and neck position. However, this is the same positioning as was used in our laboratory’s previous studies of peripharyngeal tissue pressure in rabbits and was included in the experimental design to maintain a basis for comparison between studies (4–6).

During the reformating of the CT images, axial slice thickness obtained using CT varied between the rabbits by up to 0.2 mm. This may have affected the analysis of cross-sectional area because the image data-averaging process may have varied slightly between rabbits. Thus there is some potential for reduced precision in reporting actual lumen cross-sectional area for any particular level within the upper airway.

This study examined the effect of an acute increase in tissue volume, which may not be representative of what happens in OSA, where tissue increases occur in a chronic fashion. In a monkey model in which tissue volumes were chronically increased in the lateral pharyngeal walls via collagen injections, sleep-disordered breathing was induced (10). This suggests that the impact on the upper airway of acute and chronic tissue volume increases are likely to be similar.

The site of tissue expander balloon insertion will likely influence any resulting impact on upper airway anatomy and function; thus results may differ if other sites were tested. We assessed upper airway patency using both anatomic (pharyngeal airway lumen cross-sectional area using CT) and functional (Rua) metrics. Consequently, we were able to both localize the site of airway narrowing and assess its functional outcome. It should be noted that we did not directly measure effects on upper airway collapsibility, although the development of inspiratory flow limitation in some rabbits with tissue expander inflation suggest that increased collapsibility also occurred.

**Effects of acute increases in peripharyngeal tissue volume.**

The effect of lateral pharyngeal fat pad expansion on upper airway size and Rua has been previously demonstrated by Winter et al. (21). These workers inflated balloon occlusion catheters with saline in the lateral pharyngeal fat pads of pigs, and they demonstrated that with loads as small as 4 ml of saline there was a reduction in retropalatal airway size that was associated with an increase in Rua (21). However, although a change in peripharyngeal tissue pressures was hypothesized, it was not actually measured in these studies. Our study now confirms that an acute increase in peripharyngeal tissue volume is associated with a local increase in upper airway extraluminal tissue pressure.

In a study of the effect of application of external loads to the anterior aspect of the rabbits neck, loads of 10 g or more have been demonstrated to increase inspiratory Rua by ~20%, a value similar to the ~18% increase in the present study (7). However, in our study, these resistance changes were obtained with tissue expansion volumes of only 1.5 ml of saline, (equivalent to 1.5 g of fat). Thus, by placing the load internally rather than externally, a similar effect is achieved with a much smaller load.

In this study, we also demonstrated that there is a nonuniform topographical peripharyngeal tissue pressure response to acute increases in peripharyngeal tissue volume because anterolateral tissue pressures increased but anterior tissue pressures did not. This finding suggests that there is some compartmentalization of the peripharyngeal tissue pressures.

The changes in cross-sectional area with loading occurred even above the rostral position of the tissue expander. This suggests that the upper airway is relatively incompressible in the longitudinal direction. If a force is applied to a structure that is highly compliant in the longitudinal direction, there will be local deformation but no little or no change in cross-sectional area above or below the compression. If a force is applied to a structure that is relatively noncompliant in the longitudinal direction, such as a flexible piece of bamboo, it will bow, with changes in cross-sectional area occurring above and below the site of the application of the force.

In human subjects, there has been a great deal of interest in the contribution of the lateral pharyngeal walls to the pathophysiology of OSA. An increase in thickness and volume of the lateral pharyngeal walls is associated with an increase in the risk of OSA (13, 15) and demonstrates familial aggregation (12). Indeed, expansion of the lateral wall using collagen injections results in the development of OSA in a monkey model (10). If our findings in rabbits are applicable to humans, it may be that the increased volume of tissue (e.g., large fat pads) in the lateral pharyngeal walls of patients with OSA (9, 13–15) may be associated with increased local peripharyngeal tissue pressure and decreased upper airway patency.

**Conclusion.** We have demonstrated that an acute increase in anterolateral peripharyngeal tissue volume results in an increase in anterolateral, but not anterior, peripharyngeal tissue pressures and that it is associated with a reduction in local airway cross-sectional area and an increase in overall Rua. In subjects with OSA, who may have increased peripharyngeal tissue volumes, localized increases in tissue pressure may...
impact on upper airway patency and contribute to the pathogenesis of OSA.

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