Periphrangeal tissue deformation and stress distributions in response to caudal tracheal displacement: pivotal influence of the hyoid bone?

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Amatoury J, Kairaitis K, Wheatley JR, Bilston LE, Amis TC. Periphrangeal tissue deformation and stress distributions in response to caudal tracheal displacement (TD) leads to improvements in upper airway (UA) function and decreased collapsibility. To better understand the mechanisms underlying these changes, we examined effects of TD on periphrangeal tissue stress distributions [i.e., extraluminal tissue pressure (ETP)], deformation of its topographical surface (UA lumen geometry), and hyoid bone position. We studied 13 supine, anesthetized, tracheostomized, spontaneously breathing, adult male New Zealand white rabbits. Graded TD was applied to the cranial tracheal segment from 0 to 10 mm. ETP was measured at six locations distributed around/along the length of the UA, covering three regions: tongue, hyoid, and epiglottis. Axial images of the UA (nasal choanae to glottis) were acquired with computed tomography and used to measure lumen geometry (UA length; regional cross-sectional area) and hyoid bone displacement. TD resulted in nonuniform decreases in ETP (generally greatest at tongue region), ranging from −0.07 (−0.11 to −0.03) [linear mixed-effects model slope (95% confidence interval)] to −0.27 (−0.31 to −0.23) cmH2O/mm TD, across all sites. UA length increased by 1.6 (1.5–1.8)%/mm, accompanied by nonuniform increases in cross-sectional area (greatest at hyoid region) ranging from 2.8 (1.7–3.9) to 4.9 (3.8–6.0)%/mm. The hyoid bone was displaced caudally by 0.22 (0.18–0.25) mm/mm TD. In summary, TD imposes a load on the UA that results in heterogeneous changes in periphrangeal tissue stress distributions and resultant lumen geometry. The hyoid bone may play a pivotal role in redistributing applied caudal tracheal loads, thus modifying tissue deformation distributions and determining resultant UA geometry outcomes.

upper airway; tissue pressure; tracheal traction; lung volume; pharynx

PHARYNGEAL AIRWAY LUMEN SIZE and collapsibility are known to be influenced by changes in lung volume. Increasing lung volume promotes reduced pharyngeal collapsibility and increased pharyngeal airway lumen size (9, 17, 22, 35, 36). Caudal displacement (or traction) of the trachea is thought to be the primary mechanism mediating these interactions between the lower and upper airways (41, 42).

Previous studies that have examined the mechanical consequences of caudal tracheal displacement have largely modeled the upper airway as a Starling resistor, modeling the upper airway as a Starling resistor has limitations. For instance, the behavior of the pharyngeal tissues is lumped and described by a single fixed parameter (i.e., extraluminal tissue pressure). This oversimplification ignores mechanical interactions within the periphrangeal tissues, the topographical surface of which forms upper airway lumen walls. Our laboratory has previously investigated the complex and nonuniform behavior of periphrangeal tissues in anesthetized animals by examining tissue pressures, i.e., extraluminal tissue pressure (ETP) (24–29). More recently, anesthetized animal (4, 5) and awake human studies (7, 8, 11, 12) have approached this question by measuring periphrangeal tissue deformation (strain). Such studies support a model that includes nonuniform tissue behavior (in both space and stiffness) to better describe the mechanics of the upper airway.

In this study, we are extending current concepts based primarily on airway pressures with a consideration of mechanics of the airway walls themselves. This approach aligns with the concepts suggested by the hydrostat model of the upper airway (23). In this model, the mechanical consequences of an intervention are determined by redistribution of periphrangeal tissue pressures, which in turn alters the shape of the pharyngeal tissue mass and, in consequence, upper airway lumen geometry, i.e., through changes in transmural pressures (intraluminal pressure – ETP). However, an additional interpretation of tissue pressure arises in engineering that relates it to the mechanical characteristics of tissue. That is, tissue pressure can be expressed in terms of tissue stress (a measure of the internal forces within a tissue at a point), or, more specifically, a measure of the mean compressive stress in a given area, where ETP = −stress (16, 31, 38). The tissue stresses include contributions not only from applied pressures, such as those at the airway wall, but also from muscle contraction and tissue stretching. The magnitude and distribution of stresses is fundamental to understanding the behavior of structures and are also important in physiology, e.g., ventricular wall stress distributions influence myocardial oxygen consumption and ventricular remodeling (20, 49).

An early investigation of how tracheal displacement influences pharyngeal airflow suggested that movement of the hyoid bone may play a key role (41). The hyoid bone is a mobile structure with mechanical attachments to the thyroid cartilage, which itself is likely to move with tracheal displacement. Furthermore, the hyoid bone anchors several pharyngeal muscles, providing it with the ability to redistribute applied loads throughout the periphrangeal tissue mass. Many studies have largely ignored the role of the hyoid bone (24, 32, 37), and its movement with tracheal displacement was previously...
only speculated, i.e., no direct measurement or only qualitative observation (41). Thus quantitative confirmation of hyoid movement with tracheal displacement is required to further elucidate the hyoid bone’s influence on the upper airway.

We have previously demonstrated in an animal model that caudal tracheal displacement nonuniformly reduces peripharyngeal tissue pressures (24). However, in these studies, peripharyngeal tissue pressure measurement was limited to only two sites in upper airway wall submucosa, and there were no simultaneous measurements of upper airway lumen geometry or hyoid bone movement.

The aim of the present study was to utilize an anesthetized rabbit model to examine the effects of caudal tracheal displacement on the following: 1) peripharyngeal tissue pressures at several locations distributed throughout the peripharyngeal tissue mass; 2) upper airway lumen geometry; and 3) displacement of the thyroid cartilage and hyoid bone.

**METHODS**

**Subjects**

Studies were performed in 13 adult male New Zealand White rabbits [weight = 3.7 ± 0.6 kg (mean ± SD)]. The protocol was approved by the Sydney West 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 then maintained with a continuous intravenous infusion (ear vein) of ketamine (15 mg·kg\(^{-1}\)·h\(^{-1}\)) and xylazine (4.5 mg·kg\(^{-1}\)·h\(^{-1}\)). Anesthesia levels were titrated to suppress individual rabbit upper airway muscle activity as monitored by sternohyoid muscle electromyogram (EMG; see below). Rabbis were maintained in a physiologically stable state throughout the study, as determined by monitoring heart and respiratory rate. Animals were euthanized at the completion of each study using an overdose of intravenous pentobarbital sodium (10 ml).

**Experimental Setup**

Rabbits were studied supine, and head/neck position was controlled such that a line drawn from the tragus to the external nares was at 50° to the horizontal (Fig. 1). The trachea was surgically exposed and then transected between the third and fourth cartilaginous rings. The cranial and caudal tracheal segments were cannulated separately, and rabbits were allowed to breathe spontaneously through the caudal trachea. The cranial tracheal segment was reextended to pretranssection position using the tracheal displacement apparatus (see below).

**Monitoring**

EMG. For EMG monitoring, bipolar, Teflon-coated stainless steel wire electrodes (0.08 mm bare, 0.14 mm coated; SDR Technology, Sydney, NSW, Australia) were inserted into the belly of the left (n = 8) or right (n ≥ 5) sternohyoid muscle and connected to an amplifier system (Neotrace NT 1990; Neomedix Systems, Sydney, NSW, Australia).

Tracheal pressure and flow. A heated pneumotachograph (Fleisch 8300A, 0–2 l/min range; Hans Rudolph, Kansas City, MO), with differential pressure transducer (Validyne DP45–32; Validyne Engineering, Northridge, CA), was connected to the caudal tracheal cannula to monitor inspiratory and expiratory airflow. A separate pressure transducer (Celesco LCVR ±10 cmH\(_2\)O; Celesco Transducer Products, Cango Park, CA) was connected to the caudal tracheal segment to monitor tracheal intraluminal pressure.

**Measurement of ETP**

ETP (cmH\(_2\)O) was measured simultaneously at six locations surrounding the upper airway using pressure transducer-tipped catheters (Millar SPR-524; Millar Instruments, Houston, TX) with associated control units (Millar PCU-2000; Millar Instruments).

Upper airway regions and ETP measurement locations. Three regions along the length of the upper airway were targeted for insertion of pressure transducer-tipped catheters (Fig. 1): R1 (tongue region), extending from the nasal choanae to the superior surface of the hyoid bone; R2 (hyoid region), extending from the hyoid bone to 1.5 mm above the superior surface of the epiglottis; and R3 (epiglottis region), extending from above the epiglottis tip (base of R2) to the glottis. Two ETP measurements were acquired from each region, at tissue positions anterior and lateral to the upper airway lumen.

Catheters were surgically inserted through the ventral neck to depths ranging between 20 and 25 mm for R1; 15 and 20 mm for R2; and 5 and 10 mm for R3. Pressure transducer-tipped catheters were inserted via saline-filled cannulae (16 G), and sutured in place (5.0 prolene) at the level of the submucosa and skin. Catheter (sensor) positions were determined post hoc from acquired image data (see below).

**Application of Caudal Tracheal Displacement**

Graded caudal displacement was applied to the cranial trachea using a custom-made calibrated screw mechanism attached via a tensile thread (suture; 5.0 prolene) to the cranial tracheal cannula.
Imaging

Computed tomography (CT) imaging (Toshiba Aquillion 16; Toshiba, Tokyo, Japan) was used to obtain axial images from the tip of the nares to at least 20 mm below the caudal end of the cranial tracheal segment [slice thickness = 0.4 mm (n = 2) or 0.3 mm (n = 11); resolution = 0.2 × 0.2 mm; 512 × 512 matrix].

Protocol

Caudal displacement of the trachea was applied in ~1.25-mm increments from 0 to 5 mm and then in ~2.5-mm increments from 5 to 10 mm, except for two rabbits where displacements were applied in ~2.5-mm increments over the 0- to 10-mm range. Each level of displacement was held constant for an ~10-s period during which steady-state physiological signals were recorded continuously and CT data acquired. The protocol was repeated three times, with the trachea returned to the initial position before each run.

All physiological signals were sampled at 4 kHz and digitized with a 16-bit, 16-channel analog-to-digital converter (Powerlab 16/30; ADInstruments, Sydney, NSW, Australia).

Data Analysis

ETP. ETP sensor location. Before analysis, ETP sensor location was verified from the baseline CT image set (tracheal displacement = 0 mm). Sensor positions were defined in three locations for each upper airway region: anterior (i.e., ETPAnt), anterolateral (i.e., ETPAntLat), and lateral (i.e., ETPLat). Measurements were excluded if sensors were too far from the upper airway lumen (>10 mm beyond lumen boundaries; n = 4) or in direct contact with another catheter (n = 3).

ETP analysis. Average ETP values at each location were obtained for each level of tracheal displacement. If two sensors occupied the same area within a region in an individual rabbit, they were considered as one measurement, and their values averaged (n = 10). Data were expressed as absolute change in ETP from baseline (ΔETP, cmH2O).

Image processing. At each tracheal displacement increment, axial cross sections of the upper airway lumen and hyoid bone were analyzed as follows. The process is summarized in Fig. 2.

Fig. 2. A: axial CT images are shown at the level of the tongue and hyoid bone [Ant (A), posterior (P), right (R), and left (L) orientation] illustrating segmentation of the peripharyngeal tissue surface (striped fill). B: segmentation of each axial slice, within an image set, results in a stack of axial lumen contours, i.e., axial cross-section outlines (shown in perspective). C: the peripharyngeal tissue surface was then reconstructed from the axial contours. The surface reconstruction was capped at upper and lower openings to form a solid. D: the midsagittal contour was formed by the intersection of the solid with the midsagittal (XY) plane.

UPPER AIRWAY LUMEN SEGMENTATION. The upper airway lumen was segmented from the level of the nasal choanae to the base of the epiglottis (Amira version 5.2; Visage Imaging, San Diego, CA) using Hounsfied units (HU) ranging between ~1,024 and ~150 HU (Fig. 2A). Luminal contours were created and smoothed (Fig. 2B). Subsequent downsampling of upper airway cross sections was undertaken, leaving every third (n = 2) or fourth (n = 11) contour (slice thicknesses of 0.4 or 0.3 mm, respectively), i.e., 1.2-mm spacing between contours. Contours were exported for subsequent reconstruction and analysis.

UPPER AIRWAY LUMEN RECONSTRUCTION AND MIDSAGITTAL CONTOUR. Peripharyngeal tissue surface topography (i.e., pharyngeal lumen) was reconstructed from segmented axial contours using Rhinoceros version 4 (Robert McNeel and Associates, Seattle, WA) (Fig. 2C). A midsagittal contour was created from the closed curve formed by the intersection of the pharyngeal lumen reconstruction (capped at both ends) and the midsagittal (XY) plane (Fig. 2D).

HYOID BONE SEGMENTATION. In a similar process, axial contours of the hyoid bone were obtained using a range of 226 to 3,071 HU. Upper airway lumen geometry analysis. From the upper airway lumen reconstructions and segmented contours, the following measurements were made: 1) length, perpendicular distance between upper and lower boundaries of the upper airway lumen reconstruction; 2) volume; 3) midsagittal (cross-sectional) area (MSA), from the midsagittal lumen contour; 4) axial cross-sectional area (CSA), from each axial lumen contour; 5) anteroposterior diameter (APD) and lateral diameter (LD), length of APD and LD passing through the centroid point of each axial contour.

For each upper airway region (R1, R2, and R3), the mean value of each axial geometry metric (CSA, APD, LD) was calculated from the contours of each region. All lumen geometry data were expressed as percent change from baseline. Baseline region definitions were maintained for R1 and R2 at each tracheal displacement increment. However, to accommodate for any change in upper airway length, the caudal boundary of R3 (i.e., glottis) was reselected at each tracheal displacement level. Data were excluded if the upper airway lumen was completely closed over one or more regions at baseline.
Measured tracheal displacement levels. Tracheal displacements were measured from corresponding axial CT images. A fixed point at the caudal base of the cranial tracheal segment was identified, and its coordinate obtained at each tracheal displacement level using image analysis software (Amira version 5.2). The magnitude of the resultant vector displacements and angles were calculated.

Thyroid cartilage displacement. A fixed point at the cranial tip of the thyroid cartilage was identified from axial images, and its coordinate obtained using image analysis software (Amira version 5.2) at each load increment. Both the component vectors [i.e., X (anteroposterior axis), Y (cranial-caudal axis), and Z (lateral axis)] and magnitudes of the resultant vector displacements [i.e., \( \sqrt{X^2 + Y^2 + Z^2} \)] and angles were calculated.

Hyoid bone displacement. Hyoid bone displacement was calculated as the displacement from baseline of the volumetric centroid position of the hyoid bone at each load increment. Component and resultant hyoid bone displacement vectors were then obtained in a similar manner to thyroid cartilage displacements.

Statistical Analysis

Linear mixed-effects modeling was used to examine relationships between caudal tracheal displacement and outcome metrics (ETP, upper airway lumen geometry, thyroid cartilage, and hyoid bone displacements). Rabbit identifier and tracheal displacement were considered as random effects, whereas all analyzed outcomes were considered as fixed effects. Model results were expressed as slope (95% confidence interval). Bonferroni corrections were applied for multiple comparisons where appropriate. \( P < 0.05 \) was considered significant.

RESULTS

EMG Activity

No electromyographic activity was evident throughout the protocol.

Measured Tracheal Displacement

Increments of caudal tracheal displacement were 0, 1.2 \( \pm \) 0.3 (mean \( \pm \) SD), 2.6 \( \pm \) 0.5, 3.9 \( \pm \) 0.5, 5.2 \( \pm \) 0.7, 7.3 \( \pm \) 0.8, and 8.9 \( \pm \) 0.9 mm at an angle of 24 \( \pm \) 4\(^\circ\) to the horizontal (i.e., approximate natural angle of rabbit trachea in the current setup).

ETP

Six measurements of ETP were acquired in four rabbits, five in two rabbits, four in six rabbits, and three in one rabbit. Across all sites and rabbits, ETP catheter sensor tips were located between 1 and 10 mm from the border of the upper airway lumen.

In general, baseline ETP values were above atmospheric, and greater at cranial (R1) upper airway positions compared with mid- (R2) and caudal (R3) positions. Fluctuations coinciding with frequency of respiration were evident at the majority of locations (Fig. 3).

Caudal tracheal displacement decreased ETP from baseline at all measured sites, except at the anterior position in R3 (Figs. 3 and 4). There was a significant three-way interaction between region, position, and tracheal displacement on \( \Delta \text{ETP} \) (cmH\textsubscript{2}O) \( (P < 0.001) \), i.e., nonuniform decrease in ETP throughout the upper airway lumen mass (Fig. 4; see Table 1 for values). The decrease in both ETP\textsubscript{Ant} and ETP\textsubscript{AntLat} with tracheal displacement was greatest at R1, followed by R2 and then R3 \( (P < 0.01, \text{all comparisons}) \). The decrease in ETP\textsubscript{Lat} was greatest at R1 and R3 \( (P < 0.001, \text{R1 and R3 vs. R2; } P > 0.2, \text{R1 vs. R3}) \). At R1, the greatest decrease in ETP was for ETP\textsubscript{AntLat} \( (P < 0.005) \), while the greatest decrease at R2 and R3 was for ETP\textsubscript{AntLat} and ETP\textsubscript{Lat} \( (P < 0.001) \), ETP\textsubscript{AntLat} and ETP\textsubscript{Lat} vs. ETP\textsubscript{Ant; } P > 0.2, ETP\textsubscript{AntLat} vs. ETP\textsubscript{Lat} \) (Table 1).

Upper Airway Lumen Geometry

Upper airway lumen geometry was analyzed in nine rabbits. Four rabbits were excluded due to upper airway collapse.

Length, volume, and MSA. Upper airway length and volume increased in all rabbits by 1.6 (1.5–1.8) and 6.2 (5.0–7.3)%/mm caudal tracheal displacement, respectively (both \( P < 0.001 \); Fig. 5). MSA also increased with tracheal displacement \[3.9 (3.3–4.6)%/mm; \( P < 0.001 \)].

Axial lumen geometry (CSA, APD, and LD). CSA (Fig. 6), APD, and LD increased in all rabbits with caudal tracheal displacement, except at R3 for two rabbits. Axial lumen geometry changes with tracheal displacement were nonuniform, i.e., there was a significant interaction between region and tracheal displacement on the rate of change in CSA, APD, and LD per millimeter of tracheal displacement \( (P < 0.001, \text{all interactions}) \). The greatest increases in CSA, APD, and LD with caudal tracheal displacement occurred at R2 \( (P < 0.005) \), R2 vs. R1 and R3 for each metric, except APD where \( P > 0.1 \) for R2 vs. R1), with similar increases occurring at R1 and R3 for corresponding metrics \( (P > 0.2, \text{R1 vs. R3 for each metric}) \) (Table 2, Fig. 6).

Thyroid Cartilage Displacement

Caudal tracheal displacement resulted in large thyroid cartilage displacements in the caudal-anterior (\(-Y, +X\) direction (Table 3). The resultant thyroid cartilage displacement was 0.62 (0.50–0.74) mm for every millimeter of caudal tracheal displacement, at an average angle of 155 \( \pm \) 5\(^\circ\) (mean \( \pm \) SD) to the cranial (+Y) axis (within the XY, midsagittal, plane; \( P < 0.001, \text{both values}) \).

Hyoid Bone Displacement

Caudal tracheal displacement resulted in predominantly caudal (\(-Y\) displacement of the hyoid bone in all rabbits (Fig. 7, Table 3). The resultant hyoid bone displacement was 0.22 (0.18–0.25) mm/mm caudal tracheal displacement, at an average angle of 174 \( \pm \) 5\(^\circ\) to the +Y-axis (within the XY plane; \( P < 0.001, \text{both values}) \).

DISCUSSION

In this study, caudal tracheal displacement nonuniformly decreased ETP, lengthened the upper airway, nonuniformly increased upper airway lumen size, and displaced the hyoid bone in a caudal direction.

Critique of Methods

Passive upper airway. Upper airway muscles were quiescent throughout application of tracheal displacement, as indicated by the absence of sternohyoid muscle electromyographic activity; thus results of the present study represent the passive
Fig. 3. Raw data from one rabbit demonstrating the effect of increasing levels of caudal TD (0–9.2 mm) on extraluminal tissue pressure (ETP) at measured positions (Ant/AntLat) within each upper airway region (R1, R2, R3). Note that, as caudal TD is applied, ETP decreases at all locations, except ETPAnt in R3. Note respiratory fluctuations in all ETP measurements and the heterogeneity in respiratory phasing among locations. Pr, caudal tracheal pressure, measured together with airflow at the caudal tracheal segment; EMG, electromyographic activity from the sternohyoid muscle; au, arbitrary units.
mechanical properties of the upper airway. Upper airway muscle recruitment will likely modify reported outcomes.

**ETP measurement.** The majority of ETP measurements reported in the literature are based on the methodological approach of Kairaitis et al. (24–29), who surgically implanted transducers directly into the upper airway submucosal tissues of rabbits. Limitations of this methodology have been discussed elsewhere (24–29). The present study measured pressures within the peripharyngeal tissue mass itself. Furthermore, a greater number of pressure measurements were made in the present study compared with previous studies (24–29, 48). The pressure measurements were distributed throughout the entire tissue mass, and positioning confirmed with imaging. A potential limitation of tissue pressure measurement in the present study is that sensors were located at different distances from the airway lumen at individual ETP measurement sites in different rabbits. This may have introduced variability in ETP changes with tracheal displacement within a pooled measurement location. Furthermore, it should be considered that the insertion of six catheters into the peripharyngeal tissue space of a rabbit may have altered tissue deformation patterns, and thus outcomes may not necessarily represent the situation without catheters in situ.

Fig. 4. Individual rabbit data (different symbols) and linear mixed-effects model (line) showing the change (Δ) in ETP from baseline (dotted line = 0 cmH₂O) with caudal TD at each position (displayed horizontally), i.e., Ant (A–C), anterolateral (AntLat; D–F), and lateral (Lat; G–I), within each upper airway region (displayed vertically): R1 (A, D, and G), R2 (B, E, and H), and R3 (C, F, and I). TD results in a decrease in ΔETP at each position (except R3 Ant; C) for each region, with R1 > R2 > R3. *p < 0.001. NS, not significant.
Table 1. Change in ETP per millimeter of caudal tracheal displacement

<table>
<thead>
<tr>
<th>Region</th>
<th>Position</th>
<th>ΔETP, cmH2O/mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>Ant</td>
<td>-0.17 (-0.20 to -0.13)</td>
</tr>
<tr>
<td></td>
<td>AntLat</td>
<td>-0.27 (-0.31 to -0.23)</td>
</tr>
<tr>
<td></td>
<td>Lat</td>
<td>-0.09 (-0.13 to -0.05)</td>
</tr>
<tr>
<td>R2</td>
<td>Ant</td>
<td>-0.07 (-0.10 to -0.04)</td>
</tr>
<tr>
<td></td>
<td>AntLat</td>
<td>-0.11 (-0.14 to -0.08)</td>
</tr>
<tr>
<td></td>
<td>Lat</td>
<td>-0.10 (-0.14 to -0.07)</td>
</tr>
<tr>
<td>R3</td>
<td>Ant</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>AntLat</td>
<td>-0.07 (-0.11 to -0.03)</td>
</tr>
<tr>
<td></td>
<td>Lat</td>
<td>-0.08 (-0.13 to -0.04)</td>
</tr>
</tbody>
</table>

Values are linear mixed-effects model slope, with 95% confidence interval (CI) in parentheses. ΔETP, change in extraluminal tissue pressure; R1, extending from the nasal choanae to the superior surface of the hyoid bone; R2, extending from the hyoid bone to 1.5 mm above the superior surface of the epiglottis; R3, extending from the base of R2 to the glottis (base of epiglottis); Ant, anterior; AntLat, anterolateral; Lat, lateral; NS, not significant. All slope values \( P < 0.001 \).

**Image analysis.** Analysis of upper airway lumen geometry was performed from axial CT images with all slices perpendicular to the horizontal axis, in a similar manner to several earlier studies (9, 15, 30, 43). To the extent that the upper airway is curved, this approach runs the risk of obtaining cross-sectional data at varying angles to the curved upper airway centroid axis. Changes in curvature during the intervention could, therefore, introduce bias in measurements. However, the longitudinal curvature of the rabbit upper airway is minimal, and inspection of the CT data indicated that this curvature was unchanged by the tracheal displacement, reducing the potential impact of this issue.

**Statistics.** A power calculation to determine sample size was not undertaken before the study. However, a post hoc power calculation for our primary outcomes (between region comparisons) revealed that, wherever the Bonferroni corrected \( P \) value was significant \( (P < 0.05) \), the associated power for any particular comparison was >90%. Exceptions were R2 vs. R3 for ΔCSA and ΔAPD, with 68 and 75% power, respectively.

**Respiratory-related ETP Fluctuations**

Following transection of the trachea, respiratory-related fluctuations in ETP were still present (see Fig. 3), likely resulting from transmission of forces through remaining soft tissue connections of the neck, such as the strap muscles (41). However, differences in respiratory phasing were evident between different tissue sites. We speculate that heterogeneity of respiratory phasing may reflect the complexity of the vector transmission of forces throughout the peripharyngeal tissues, particularly once the dominant inspiratory caudal tracheal displacement effect has been removed.

**Caudal Tracheal Displacement and ETP**

Graded application of caudal tracheal displacement decreased ETP at all measurement locations (except anterior position at the epiglottis region, R3), with the amount of reduction being greatest at rostral and anterolateral peripharyngeal tissue regions (i.e., R1 > R2 > R3). Our laboratory has previously reported a decrease in ETP with increasing caudal tracheal displacement (24), measured from anterior and lateral positions at a region approximately equivalent to R2 in the present study. Despite slight differences in ETP measurement methodology, the decreases in ETP at corresponding positions between studies were similar in magnitude (24).

ETP has conventionally been associated with transmural pressure (\( P_{\text{transmural}} = P_{\text{intraluminal}} - \text{ETP} \)) in determining upper airway patency (18, 19, 26, 32). However, ETP can also be applied to describe the mechanical characteristics of tissue when considered in terms of stress. An ETP-stress relationship emerges when dividing stress into components that act to alter tissue volume and those that alter tissue shape, i.e., mean and shear stress, respectively (16). The volumetric mean stress \( (\sigma_m) \) is calculated as the average of the stresses in three orthogonal directions \((\sigma_x, \sigma_y, \sigma_z)\), i.e.

\[
\sigma_m = \frac{\sigma_x + \sigma_y + \sigma_z}{3}
\]

As such, the mean stress is considered a hydrostatic stress (i.e., equally exerted in orthogonal directions), and from the theory of elasticity can be re-interpreted as a measure of the internal hydrostatic pressure, or more specifically ETP, that is exerted by a state of stress (38), such that:

\[
\sigma_m = -\text{ETP}
\]

or,

![Fig. 5. Individual rabbit data (different symbols) and linear mixed-effects model (line) showing the percent change in upper airway length (ΔLength; A) and volume (ΔVolume; B) from baseline with caudal TD (dotted line = 0%). TD increased both length and volume in all rabbits (\( P < 0.001 \) for model slopes).](http://jap.physiology.org)
The impact of caudal tracheal displacement in anesthetized pigs

Two previous studies have examined airway lumen size (i.e., CSA, APD, and LD) with caudal analysis techniques to show that there is an increase in upper airway lumen geometry and an apparent increase in tissue stiffness, such that a decrease in ETP indicates an increase in tissue stress (or increased apparent stiffness, and vice versa). When interpreted within this paradigm, the magnitude (and direction; positive or negative) of the ΔETP can provide an indication of how externally applied loads (such as tracheal displacement/lung volume) influence local peripharyngeal tissue stiffness distributions.

Caudal Tracheal Displacement and Upper Airway Lumen Geometry

This is the first study to use sophisticated imaging and analysis techniques to show that there is an increase in upper airway lumen size (i.e., CSA, APD, and LD) with caudal tracheal displacement. Two previous studies have examined the impact of caudal tracheal displacement in anesthetized pigs at a single pharyngeal cross section using video-endoscopy (39, 40). These studies showed no consistent/significant changes in upper airway lumen size with tracheal displacement across all regions (39). Potential reasons for these different outcomes include different imaging methodologies and species. In particular, the pig, unlike rabbits (2, 14) and humans, has an anatomically different hyoid bone (or hyoid apparatus, consisting of an articulated chain of bones) that is fixed to the base of the skull. The difference in hyoid anatomy may alter the interactions between caudal tracheal displacement and upper airway lumen geometry.

The increase in upper airway lumen dimensions with caudal tracheal displacement in rabbits is likely to be associated with a fall in upper airway resistance. Previously published studies in paralyzed cats demonstrated either no change (37) or a significant increase in upper airway resistance (32) with caudal tracheal displacement. This may be again explained by a reduced range of movement of the hyoid bone in cats, which, similar to pigs, articulates with the skull base (47). Direct application of tracheal displacement has not been studied in humans, but increasing lung volume, which causes caudal tracheal displacement (28, 41), has been shown to increase pharyngeal CSA (9, 17) and decrease upper airway resistance during both wakefulness (33) and sleep (35). In addition,

\[ \text{ETP} = -\sigma_m \]

From this relationship, a reduction in ETP indicates an increase in tissue stress (\( \downarrow \text{ETP} = \uparrow \text{stress} \)), and vice versa (10, 50). When measured from within the peripharyngeal tissue mass [rather than the submucosal space, as with previous measurements (24–29)], changes in ETP can be interpreted as reflecting changes in the distribution of local tissue mechanics. In general, soft biological tissues are considered incompressible (volume conserving) and hyperelastic, such that the stress increases with increasing strain (change in length per unit length) (16). The slope of the stress-strain relationship at any point is the elasticity or stiffness of the tissue. Thus, in the case of a hyperelastic material, a change in stress indicates a change in apparent tissue stiffness, such that \( \uparrow \text{stress} \) (or \( \downarrow \text{ETP} \) = \( \uparrow \) apparent stiffness, and vice versa. When interpreted within this paradigm, the magnitude and direction; positive or negative) of the ΔETP can provide an indication of how externally applied loads (such as tracheal displacement/lung volume) influence local peripharyngeal tissue stiffness distributions.

### Table 2. Change in axial upper airway lumen geometry metrics per millimeter of caudal tracheal displacement

<table>
<thead>
<tr>
<th>Region</th>
<th>ΔCSA</th>
<th>ΔAPD</th>
<th>ΔLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>2.8 (1.7–3.9)</td>
<td>1.7 (1.1–2.4)</td>
<td>0.9 (0.5–1.3)</td>
</tr>
<tr>
<td>R2</td>
<td>4.9 (3.8–6.0)*</td>
<td>2.3 (1.7–3.0)</td>
<td>2.2 (1.9–2.6)*</td>
</tr>
<tr>
<td>R3</td>
<td>3.1 (2.0–4.2)</td>
<td>1.4 (0.7–2.0)</td>
<td>1.0 (0.6–1.4)</td>
</tr>
</tbody>
</table>

Values are linear mixed-effects model slope, with 95% CI in parentheses, in %/mm. CSA, cross-sectional area; APD, anteroposterior diameter; LD, lateral diameter. All slope values \( P < 0.001 \). *\( P < 0.001 \) vs. R1 and R3 for corresponding metrics (Bonferroni corrected for multiple comparisons).

### Table 3. Thyroid cartilage and hyoid bone displacements per millimeter of caudal tracheal displacement

<table>
<thead>
<tr>
<th>Thyroid Cartilage Displacement</th>
<th>Hyoid Bone Displacement</th>
</tr>
</thead>
<tbody>
<tr>
<td>X (AP), mm/mm</td>
<td>0.22 (0.18–0.24)</td>
</tr>
<tr>
<td>Y (CC), mm/mm</td>
<td>−0.59 (−0.65 to −0.53)</td>
</tr>
<tr>
<td>Z (lateral), mm/mm</td>
<td>NS</td>
</tr>
<tr>
<td>Magnitude, mm/mm</td>
<td>0.62 (0.50–0.74)</td>
</tr>
<tr>
<td>Angle, °</td>
<td>155 ± 5</td>
</tr>
<tr>
<td>XYZ (resultant), mm/mm</td>
<td>0.22 (0.18–0.25)</td>
</tr>
<tr>
<td>Magnitude, mm/mm</td>
<td>174 ± 5</td>
</tr>
</tbody>
</table>

Values are linear-mixed effects model slope, with 95% CI in parentheses, except for angle, which is means ± SD. AP, anteroposterior; CC, cranial-caudal. Displacement component directions: +X, anterior; +Y, cranial; +Z, lateral (right); XYZ, resultant displacement vector, with angle relative to +y-axis, within the XY (midsagittal) plane. All values \( P < 0.001 \).
similar to the present study, an interaction between lung volume and upper airway region on CSA was also observed in awake humans, whereby the change in CSA at the oropharynx and hypopharynx was larger than at the velopharynx (9).

A previous study has shown upper airway length (i.e., longitudinal upper airway strain) increases with caudal tracheal displacement; however, this included the change in tracheal length (37). The present study found that caudal tracheal displacement does not directly translate to increases in upper airway length, with ~62% of the applied tracheal displacement transferred to the upper airway (i.e., tracheal stretch likely accommodating the other 38%). In a physical collapsible tube bench model of the upper airway, a unique longitudinal upper airway strain level was found that produced conditions for optimal airflow characteristics and reduced collapsibility (1). A recent animal study also points to a threshold lung volume effect on upper airway function that may be associated with longitudinal strain levels (28). In the face of a possible optimal longitudinal upper airway strain level, the methodology of airway length measurement requires additional consideration when reviewing reported results.

Caudal Tracheal Displacement and Hyoid Bone Movement

The present study is the first to report hyoid bone displacements with tracheal displacement. The rabbit is well suited for the study of hyoid bone mechanics, since it is one of the few nonprimate with a hyoid bone similar to that of the human, i.e., freely suspended bone with a wide range of movement. Overall, caudal tracheal displacement resulted in movement of the hyoid bone in a caudal direction.

In addition to connective and cartilaginous tissue connections, a number of muscles converge onto the hyoid bone from all regions of the upper airway. This study suggests that the hyoid bone plays a key role in transferring applied loads to different areas of the peripharyngeal tissues. As a result of this, the displacement of the hyoid bone with caudal tracheal displacement is possibly a major contributor to the changes in ETP and upper airway lumen geometry found in the present study. A schematic of the hyoid’s potential role in caudal tracheal displacement load transfer is presented in Fig. 8. With caudal tracheal displacement, caudal movement of the hyoid bone likely relays tracheal loads to suprahyoid muscles, at an obtuse angle to the tracheal displacement load direction (Fig. 8). This tissue stretch stiffens the upper peripharyngeal tissues ventrally and enlarges the airway lumen through movement of the peripharyngeal tissues forming the airway in the anterior and lateral directions. Through the action of the hyoglossus muscle, the tongue is forced to tilt downwards as the hyoid bone is caudally displaced, pulling on the soft palate in the process (i.e., via palatoglossus connection) and potentially resulting in enlargement of the airway in rostral and mid-upper airway regions (R1 and R2). Indeed, the greatest increase in upper airway lumen size was in the region of the hyoid bone.

The importance of the hyoid bone in upper airway mechanics is well recognized, with human population-based studies showing an association between hyoid bone position and upper airway function, i.e., a more caudally located hyoid bone is linked to increased upper airway resistance and collapsibility and is characteristic of those with obstructive sleep apnea (OSA) (13, 21, 34, 44, 45). However, caudal movement of the hyoid bone with tracheal displacement is likely associated with a different mechanism to that causing the more inferiorly positioned hyoid bone in OSA patients. In these patients, tissue compression caused by increased soft tissue volume within the “bony box” surrounding the upper airway (i.e., mandible-maxilla enclosure) is thought to push the hyoid bone caudally (13, 46), likely reducing tension in infrahyoid muscles. On the other hand, the caudal displacement of the hyoid bone with tracheal displacement would likely increase the tension in the tissues (both supra- and infrahyoid muscles), resulting in a stiffer airway wall, and improving upper airway stability.

Fig. 8. A schematic (lateral view) demonstrating the potential manner by which the hyoid bone transfers the applied caudal TD load, to areas throughout the rabbit upper airway, at different angles to the original load direction (via stretch of connected muscles; white arrows). When caudal TD is applied, the thyroid cartilage is displaced caudally, applying a load to thyrohyoid attachments (i.e., thyrohyoid muscle, ligaments, and connective tissue). This in turn displaces the hyoid bone caudally (dashed arrow), stretching the attached hyoid bone muscles, i.e., constrictor and stylohyoid muscles in caudal-anterior direction; thyrohyoid muscle in caudal direction; and geniohyoid and mylohyoid muscles in caudal-posterior direction. In addition, the force applied to the hyoglossus lowers the tongue, which pulls on the soft palate via the palatoglossus muscle (not shown in diagram).
A Model of Peripharyngeal Tissue Mechanics

Recent magnetic resonance (MR) imaging studies in awake human subjects using specialized MR imaging protocols (i.e., SPAMM) have demonstrated local peripharyngeal tissue deformation and movement, under a number of conditions, which was shown to be different along the length of the upper airway (7, 8, 11, 12). The majority of the tissue motion in these studies was active (i.e., during inspiration). Indeed, the present study has demonstrated that even the passive behavior of the peripharyngeal tissues is heterogeneous. It was also shown in a human study that patterns of tissue movement were different in OSA compared with healthy subjects (7). In addition, a study using MR elastography suggests intrinsic differences in peripharyngeal tissue mechanical properties of OSA subjects, showing reduced genioglossus stiffness in OSA compared with control subjects with similar body mass index (3, 6). The differences in tissue deformation and mechanical properties reinforce the need to describe upper airway mechanics using a model that includes the tissue mechanical behavior.

The nonuniform decrease in tissue pressure and associated nonuniform increase in upper airway lumen size in the current study supports the primary concepts raised by the hydrostat model of the upper airway (23). The hydrostat model focuses on the pressures and their ultimate impact on upper airway lumen geometry (i.e., effect on transmural pressures). In the present study, a complementary view of the upper airway is proposed that focuses on tissue stress distributions, interpreting upper airway behavior in terms of local changes in tissue mechanics. The mechanics of the hyoid bone itself are likely to play a major role in determining peripharyngeal tissue behavior.

In conclusion, the results from the present study have reemphasized the complexity of upper airway mechanics by demonstrating the nonuniform impact of caudal tracheal displacement on peripharyngeal tissue pressures and upper airway lumen geometry. These results support the notion that a thorough understanding of upper airway function requires an analysis of tissue behavior, not just the lumen itself (e.g., closing pressures, upper airway resistance). Moreover, the present study suggests a pivotal role for the hyoid bone in influencing peripharyngeal tissue stress-strain distributions and pharyngeal lumen size with caudal tracheal displacement.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


