Peripharyngeal tissue deformation, stress distributions, and hyoid bone movement in response to mandibular advancement

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Amatoury J, Kairaitis K, Wheatley JR, Bilston LE, Amis TC. Peripharyngeal tissue deformation, stress distributions, and hyoid bone movement in response to mandibular advancement. J Appl Physiol 118: 282-291, 2015. First published December 11, 2014; doi:10.1152/japplphysiol.00668.2014.-Mandibular advancement (MA) increases upper airway (UA) patency and decreases collapsibility. Furthermore, MA displaces the hyoid bone in a cranialanterior direction, which may contribute to MA-associated UA improvements via redistribution of peripharyngeal tissue stresses (extraluminal tissue pressure, ETP). In the present study, we examined effects of MA on ETP distributions, deformation of the peripharyngeal tissue surface (UA geometry), and hyoid bone position. We studied 13 supine, anesthetized, tracheostomized, spontaneously breathing adult male New Zealand White rabbits. Graded MA was applied from 0 to \sim 4.5 mm. ETP was measured at six locations distributed throughout three UA regions: tongue, hyoid, and epiglottis. Axial computed tomography images of the UA (nasal choanae to glottis) were acquired and used to measure lumen geometry (UA length; regional cross-sectional area) and hyoid displacement. MA resulted in nonuniform decreases in ETP (greatest at tongue region), ranging from -0.11 (-0.15 to -0.06) to -0.82 (-1.09 to -0.54) cmH₂O/mm MA [linear mixed-effects model slope (95% confidence interval)], across all sites. UA length decreased by -0.5 (-0.8 to -0.2) %/mm accompanied by nonuniform increases in cross-sectional area (greatest at hyoid region) ranging from 7.5 (3.6-11.4) to 18.7 (14.9-22.5) %/mm. The hyoid bone was displaced in a cranialanterior direction by 0.42 (0.36-0.44) mm/mm MA. In summary, MA results in nonuniform changes in peripharyngeal tissue pressure distributions and lumen geometry. Displacement of the hyoid bone with MA may play a pivotal role in redistributing applied MA loads, thus modifying tissue stress/deformation distributions and determining resultant UA geometry outcomes.

upper airway; hyoid bone mechanics; mandibular repositioning; tissue pressure; pharynx

MANDIBULAR ADVANCEMENT is commonly used as a therapeutic option for obstructive sleep apnea (OSA); however, it is only successful (i.e., apnea-hypopnea index ≤ 10) in ~50% of patients (8). The underlying causes for mandibular advancement therapy failure for any individual patient remains largely unknown. In general, mandibular advancement increases upper airway lumen dimensions (12, 18, 23, 26) and reduces pharyngeal collapsibility (11, 12).

Recently, Brown et al. (4) used tagged magnetic resonance (MR) imaging to measure peripharyngeal tissue movements and deformations (strain) during awake tidal breathing in humans. These authors reported nonuniform peripharyngeal tissue behaviors in response to mandibular advancement, with striking differences in local tissue movements between healthy subjects and OSA patients (4). We have previously demonstrated, in an anesthetized rabbit model, that mandibular advancement reduces peripharyngeal tissue pressures, i.e., extraluminal tissue pressure (ETP) (14). In this study, ETP was measured in the pharyngeal submucosa (two sites), such that the reduction in ETP reflected a decrease in pharyngeal wall transmural pressure [i.e., transmural pressure = intraluminal pressure - ETP (1, 25)]. More recently we have proposed a complementary interpretation by suggesting that ETP measured within the peripharyngeal tissue mass itself reflects hydrostatic (or mean) tissue stress (i.e., ETP = -mean stress) (2) and therefore provides an indicator of local peripharyngeal tissue stress or deformation distributions. When used in conjunction with imaging information to document resultant changes in upper airway lumen geometry (or peripharyngeal tissue surface deformation), such a model provides a window for exploration of linkages between peripharyngeal tissue deformation patterns and upper airway patency.

We recently used this model to determine the impact of caudal tracheal displacement (as occurs with increases in lung volume) on peripharyngeal tissue mechanics and accompanying pharyngeal lumen geometry outcomes in anesthetized rabbits (2). An important conclusion was that hyoid bone movement in response to caudal tracheal displacement appears to play a role in the redistribution of tissue stresses and resultant regional lumen geometry changes (2). Mandibular advancement has been similarly shown to displace the hyoid bone in humans, although in a cranial-anterior direction (3, 5, 18, 28). These findings led us to consider that differing responses to mandibular advancement therapy may be due to associated individual variation in hyoid bone position and movement. We hypothesized that mandibular advancement alters the distribution of stresses within the peripharyngeal tissues, in association with cranial-anterior displacement of the hyoid bone. The aim of the present study was to utilize our anesthetized rabbit model to better understand physiological interrelationships between mandibular advancement, hyoid bone movement, peripharyngeal tissue stress distributions, and resultant pharyngeal lumen geometry changes.

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METHODS

The present study was conducted as a further intervention performed in the same animal group described in detail in our previous publication (where the intervention reported was caudal tracheal displacement) (2). Thus the overall experimental methodology has, for the most part, been previously described (2). Below we provide summary details and expand on methodologies and protocols specific to the mandibular advancement intervention itself.

Subjects and Anesthesia

Studies were performed on a total of 13 adult male New Zealand White rabbits [weight = 3.7 ± 0.6 kg (mean \pm SD)]. The protocol was approved by the Sydney West Area Health Service Animal Ethics Committee. 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). Animals were euthanized at the completion of each study using an overdose of intravenous sodium pentobarbitone (10 ml).

Experimental Setup

Rabbits were studied supine with head/neck position controlled at 50° to the horizontal (Fig. 1). The upper airway was isolated via surgical transection of the trachea (i.e., no airflow through the pharynx; rabbits breathed spontaneously via the caudal trachea). The cranial tracheal segment was re-extended to end-expiratory pretransection position.

Monitoring of sternohyoid muscle electromyogram (EMG), as well as inspiratory/expiratory pressures and airflow at the caudal tracheal segment, was performed [see Amatoury et al. (2) for details].

Measurement of ETP

ETP was measured using pressure transducer-tipped catheters (Millar SPR-524; Millar Instruments, Houston, TX). Measurements were made simultaneously from six locations distributed in anterior and lateral tissue positions along the length of the upper airway from three regions (see Fig. 1): R1 (tongue region), R2 (hyoid region), and R3 (epiglottis region).

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 gauge) and sutured in place (5.0 prolene) at the level of skin and submucosa. Sensor positions were determined post hoc from acquired image data (see below).

Preparation and Application of Mandibular Advancement

Mandibular advancement was applied using a custom-made mandibular advancement device (MAD) with a bracket design and screw mechanism attached to the lower and upper incisors (Fig. 1). Initially, the portion of the upper incisors that overlapped the lower incisors was removed using a rotary tool (Triton TRT; Triton Manufacturing and Design) with cut-off wheel attachment. To accommodate the screw, a hole was bored (3 mm drill bit) through the upper incisors (toward the maxilla) to a depth of \sim 5 mm at an angle of \sim 75° to the horizontal [the angle of intended mandibular advancement, previously found to minimize mouth opening (14)]. A small mold (acrylic self-curing resin and liquid; Lang Dental) was made to serve as an interface between the MAD base (which naturally bonded to the mold as it set) and lower incisors to allow control of device position (with screw) at an angle of \sim 75°. The MAD was secured to the lower incisors with adhesive (Loctite 401). Additional mold material was wrapped around the MAD base/lower incisors for further support. Advancement of the mandible was achieved via rotation of the screw (1 rotation = 0.45 mm screw length change).



Fig. 1. Schematic representation of the experimental setup. Rabbits were positioned supine with head/neck angle at 50° to the horizontal and the upper airway isolated (transected trachea). A custom-made mandibular advancement device (MAD) attached to the rabbits upper and lower incisors (via screw and mold, respectively) allowed graded levels of mandibular advancement to be applied at an angle of 75° to the horizontal. Peripharyngeal tissue pressure were measured between anterior [anterior extraluminal tissue pressure (ETPAnt); vertical striped circles] and lateral tissue positions [lateral extraluminal tissue pressure (ETPLat); horizontal striped circles] from three upper airway regions spanning the length of the upper airway: 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 epiglottis). Computed tomography (CT) was used to image the upper airway. Sternohyoid muscle electromyogram (EMG) was monitored.

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); in plane resolution = $0.2 \times 0.2 \text{ mm}$; $512 \times 512 \text{ matrix}$].

Protocol

Mandibular advancement was applied in \sim 0.9-mm increments from 0 to \sim 5.4 mm, except for two rabbits where advancements were applied at \sim 0, 2.7, and 5.4 mm. Each level of advancement was held constant for \sim 10 s during which steady-state physiological signals were recorded continuously and CT data acquired. The protocol was repeated three times. 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, Australia).

Data Analysis

ETP. ETP SENSOR LOCATION. Baseline CT images were used to identify the exact location of ETP sensors in each of three upper

airway regions: anterior (i.e., ETPAnt), anterolateral (i.e., ETPAnt-Lat), and lateral (i.e., ETPLat). Measurements were excluded if sensors were too far from the upper airway lumen (>10 mm beyond lumen boundaries) or in direct contact with another catheter.

ETP ANALYSIS. Average ETP values at each location were obtained for each level of mandibular advancement. 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 (Δ ETP).

Image Processing

The upper airway lumen (peripharyngeal tissue surface) was segmented from the level of the nasal choanae to base of the epiglottis (Amira v5.2; Visage Imaging, San Diego, CA) using Hounsfield units (HU) ranging between -1,024 and -150. Luminal contours were created, smoothed, downsampled (leaving a 1.2 mm gap between cross-sections), and then exported in DXF (AutoCAD Drawing Exchange File) format for subsequent analysis. In a similar process, axial contours of the hyoid bone were obtained using a range of 226–3,071 HU (with no downsampling).

A surface reconstruction of the upper airway lumen was created from segmented axial contours using Rhinoceros v4 (Robert McNeel and Associates, Seattle, WA). A midsagittal contour (i.e., outline of the peripharyngeal tissue surface in the midsagittal plane) was also created.

Image Analysis

From the upper airway lumen reconstructions and segmented axial and midsagittal contours, the following measurements to describe pharyngeal lumen geometry were made: 1 length, 2 volume, 3) midsagittal (cross-sectional) area (MSA), 4) axial cross-sectional area (CSA), 5) anteroposterior diameter (APD); and 6) lateral diameter (LD). For each upper airway region, the mean value of each axial geometry metric (CSA, APD, LD) was calculated from the contours of each region and expressed as both absolute and percent change from baseline. Data were excluded if the upper airway lumen was completely closed over one or more regions at baseline.

Actual applied mandibular advancement levels and thyroid cartilage displacements were measured from axial CT images. At each mandibular advancement increment, coordinates of fixed points at the cranial tip of both the lower incisors (for mandibular advancement measures) and thyroid cartilage were recorded using image analysis software (Amira v5.2). 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 was calculated as the change, from baseline, of its volumetric centroid (obtained from contour segmentations) at each mandibular advancement increment and similarly expressed in terms of component and resultant vector displacements.

Statistical Analysis

Linear mixed-effects modeling was used to examine relationships between mandibular advancement and all outcome metrics. Rabbit identifier and mandibular advancement were considered as random effects, while all analyzed outcomes were considered as fixed effects. Model results were expressed as slope (95% confidence interval; CI). Bonferroni corrections were applied for multiple comparisons where appropriate. P < 0.05 was considered significant.

RESULTS

EMG Activity

No electromyographic activity was observed throughout the protocol.

Measured Mandibular Advancement

The measured increments of mandibular advancement were 0, 0.7 \pm 0.3 (mean \pm SD), 1.5 \pm 0.4, 2.2 \pm 0.3, 3.0 \pm 0.3, 3.8 \pm 0.3, and 4.5 \pm 0.5 mm at an angle of 80 \pm 5° to the horizontal.

ETP

Six measurements of ETP were acquired in four rabbits, five in two rabbits, four in six rabbits, and three in one rabbit (2). Across all sites and rabbits, ETP catheter sensor tips were located between 1 and 10 mm from the border of the upper airway lumen (2).

Mandibular advancement decreased ETP from baseline at all measured sites (Figs. 2 and 3). There was a significant threeway interaction between region, position, and mandibular advancement on Δ ETP (P < 0.001), i.e., there were nonuniform decreases in ETP throughout the peripharyngeal tissue mass (Fig. 3; see Table 1 for values). Over all positions (Ant, AntLat, Lat), the decrease in ETP with mandibular advancement was greatest at R1, followed by R2 and then R3 (P < 0.001, all comparisons). For regions R1 and R2, the greatest decrease in ETP was at AntLat and/or Lat positions, while at R3 the greatest decrease occurred at the Ant position (see Table 1).

Upper Airway Lumen Geometry

Upper airway lumen geometry was analyzed in nine rabbits, with four rabbits excluded due to pharyngeal collapse [as reported previously (2)]. In addition, the last two increments of mandibular advancement were excluded in one rabbit since the oral cavity (initially closed) became visible, which made comparative measurements difficult. Upper airway lumen geometry metric values at baseline and change with mandibular advancement are presented in Tables 2 and 3.

Length, Volume, and Midsagittal Area

Upper airway length decreased slightly in most rabbits during mandibular advancement, which for the group was -0.5 (-0.8 to -0.2) %/mm of mandibular advancement (P < 0.01; Table 2, Figs. 4 and 5*A*). There were large increases in upper airway volume (Figs. 4 and 5*B*) and midsagittal area in all rabbits with mandibular advancement, i.e., 10.5 (9.4–11.5) and 6.6 (5.5–7.7) %/mm, respectively (both P < 0.001; Table 2).

Axial Lumen Geometry (CSA, APD, and LD)

CSA (Fig. 6), APD, and LD increased in all regions and rabbits with mandibular advancement. Axial lumen geometry changes were nonuniform, i.e., there was a significant interaction between region and mandibular advancement on Δ CSA, Δ APD, and Δ LD (P < 0.001, all interactions; see Table 3 for values). The greatest increases in CSA, APD, and LD with mandibular advancement occurred in the region of the hyoid bone, R2 (P < 0.001, R2 vs. R1 and R3 for all metrics; Table 3, Fig. 6). Increases were similar at tongue and epiglottis regions (R1 and R3) for CSA and LD (P > 0.1, R1 vs. R3 for both metrics; Table 3, Fig. 6), while APD increases were greater at R1 compared with R3 (P < 0.001) (Table 3).



Fig. 2. Raw data from one rabbit demonstrating the effect of increasing levels of mandibular advancement (MA; 0–4.4 mm) on extraluminal tissue pressure (ETP) at measured positions within each upper airway region: R1 (Ant/AntLat), R2 (Ant/Lat), and R3 (AntLat/Lat). Note that as mandibular advancement is applied, ETP decreases at all locations, particularly at R1. Note also respiratory fluctuations in all ETP measurements. Airflow is measured from the caudal tracheal segment, i.e., no airflow through upper airway. P_{T} , caudal tracheal pressure; EMG, electromyographic activity from the sternohyoid muscle; au, arbitrary units.

Thyroid Cartilage Displacement

Mandibular advancement resulted in small thyroid cartilage displacements in the cranial (+Y) direction, with no significant anterior or lateral movements (both P > 0.1; Table 4). The resultant thyroid cartilage vector displacement was 0.16 (0.12–0.21) mm for every millimeter of mandibular advancement, at an average angle of $0 \pm 2^{\circ}$ (mean \pm SD) to the cranial (+Y) axis (within the XY, midsagittal, plane; P < 0.001, both values).

Hyoid Bone Displacement

Mandibular advancement resulted in predominantly cranial (+Y) displacement of the hyoid bone in all rabbits, with a smaller anterior component (+X) (Fig. 7; Table 4). The resultant hyoid bone vector displacement was 0.42 (0.36-0.47) mm/mm mandibular advancement, at an average angle of 18 ±

4° to the cranial (+Y) axis (within the XY plane; P < 0.001, both values).

DISCUSSION

This study has shown for the first time that, in an anesthetized rabbit model with an isolated (passive) upper airway preparation, mandibular advancement leads to nonuniform decreases in tissue pressure around and along the length of the pharynx. In addition, this study has confirmed previous findings in humans of nonuniform increases in upper airway lumen size and displacement of the hyoid bone in a cranial-anterior direction when mandibular advancement is applied.

Critique of Methods

The broader limitations of the methodology of the present study have been described in detail previously [refer to Ama-

Fig. 3. Individual rabbit data (different symbols) and linear mixed-effects model (solid line) showing the absolute change (Δ) in ETP from baseline (dotted line = 0 cmH₂O) with mandibular advancement at each position (displayed horizontally), i.e., anterior (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*). Mandibular advancement results in a decrease in Δ ETP at all sites, with R1 > R2 > R3. *P* < 0.001 for all model slopes.



toury et al. (2)], and we primarily limit this discussion to methodological considerations specific to the mandibular advancement intervention.

The end of the cranial tracheal segment was repositioned to its pretransection position prior to commencement of the study using a custom-made apparatus (2), which limited cranial

Table 1. Change in ETP per millimeter of mandibularadvancement

Region	Position	Δ ETP, cmH ₂ O/mm
R1	Ant	-0.51 (-0.78 to -0.24)
	AntLat	$-0.82 (-1.09 \text{ to } -0.54)^*$
	Lat	-0.78 (-1.10 to -0.46)*
R2	Ant	-0.24 (-0.30 to -0.17)
	AntLat	-0.23 (-0.30 to -0.16)
	Lat	$-0.35 (-0.42 \text{ to } -0.28)^{+}$
R3	Ant	-0.18 (-0.22 to -0.14)‡
	AntLat	-0.11 (-0.15 to -0.06)
	Lat	-0.11 (-0.15 to -0.07)

Values are linear mixed-effects model slope, with 95% confidence interval (CI) in parentheses. Δ ETP, 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; Ant, anterior; AntLat, anterolateral; Lat, lateral. All slope values P < 0.001. *P < 0.03 vs. ETPAnt for R1; †P < 0.001 vs. ETPAnt and ETPAntLat for R2; ‡P < 0.001 vs. ETPAntLat and ETPLAt for R3 (all Bonferroni corrected for multiple comparisons).

movement of the trachea. However, tracheal position was not measured following application of mandibular advancement. It is possible that mandibular advancement resulted in slight cranial movements and/or stretch of the trachea, which may have influenced study outcomes.

Changes in upper airway length with mandibular advancement were limited to 1.2-mm increments due to a combination of image slice thickness and downsampling of segmented contours prior to analysis. While decreases in upper airway length were observed for most rabbits (quantitatively and also qualitatively from CT image data), the inability to detect length changes < 1.2 mm may have influenced absolute values.

Advancement of the mandible was performed at $\sim 75^{\circ}$ to the horizontal; an angle previously demonstrated to mini-

Table 2. Baseline values, absolute and percent change in upper airway length, volume, and MSA per millimeter of mandibular advancement

	Baseline	Δ	Δ , %/mm
Length	$\begin{array}{c} 41.7 \pm 3.2 \text{ mm} \\ 518 \pm 135 \text{ mm}^3 \\ 158 \pm 29 \text{ mm}^2 \end{array}$	-0.21 (-0.34 to -0.07) mm/mm	-0.5 (-0.8 to -0.2)
Volume		54.3 (42.9-65.6) mm ³ /mm	10.5 (9.4-11.5)
MSA		10.4 (8.3-12.6) mm ² /mm	6.6 (5.5-7.7)

Baseline values (MA = 0 mm) are means \pm SD. Absolute (middle column) and percent (right column) change (Δ) per mm of MA are linear mixed-effects model slopes, with 95% CI in parentheses. MSA, midsagittal cross-sectional area; MA, mandibular advancement. All slope values P < 0.001.

	Region	Baseline	Δ	Δ, %/mm
CSA	R1	$12.2 \pm 3.8 \text{ mm}^2$	1.31 (1.18–1.43) mm ² /mm	10.9 (7.0–14.7)
	R2	$9.1 \pm 4.0 \text{ mm}^{2*}$	1.80 (1.68–1.93) mm ² /mm*	18.7 (14.9-22.5)*
	R3	$15.4 \pm 4.0 \text{ mm}^2$	1.15 (1.02–1.28) mm ² /mm	7.5 (3.6–11.4)
APD	R1	$4.2 \pm 0.8 \text{ mm}$	0.28 (0.24-0.32) mm/mm ⁺	6.6 (5.4-8.3)†
	R2	$3.0 \pm 0.9 \text{ mm}^*$	0.30 (0.26-0.34) mm/mm*	10.6 (9.2–12.0)*
	R3	$4.1 \pm 0.9 \text{ mm}$	0.17 (0.13-0.21) mm/mm	4.1 (2.6–5.6)
LD	R1	3.5 ± 0.5 mm	0.10 (0.08–0.11) mm/mm	2.8 (1.7-3.9)
	R2	$3.8 \pm 0.6 \text{ mm}$	0.29 (0.27-0.30) mm/mm*	7.8 (6.7-8.9)*
	R3	$4.7 \pm 1.0 \text{ mm}$ ‡	0.12 (0.10-0.14) mm/mm	2.5 (1.3-3.6)

Table 3. Baseline values, absolute, and percent change in axial upper airway lumen geometry metrics per millimeter of mandibular advancement

Baseline values (MA = 0 mm) are means \pm SD. Absolute (middle column) and percent (right column) change (Δ) per mm of MA are linear mixed-effects model slope, with 95% CI in parentheses. CSA, axial cross-sectional area; APD, anteroposterior diameter; LD, lateral diameter. All slope values P < 0.001. *P < 0.05 vs. R1 and R3 for each metric (baseline and Δ s); $\ddagger P < 0.05$ vs. R1 and R2 for baseline LD; $\ddagger P < 0.001$ vs. R3 for Δ APD (note that baseline value region comparisons were performed using a one-way ANOVA; all comparisons were Bonferroni corrected).

mize mouth opening in rabbits (14). However, the imaging data indicates that the angle of mandibular advancement was up to 10° greater in some rabbits, i.e., 85° . This was the result of a slight tilting of the MAD screw (relative to its longitudinal axis) toward the lower incisors when the mandible was advanced. Our previous rabbit study failed to demonstrate any significant changes in either ETP or upper airway resistance between a mandibular advancement angle of 75° and 85° (14). However, experimental conditions differed between the current and previous study [i.e., isolated upper airway (no airflow) vs. intact upper airway (with airflow), respectively], raising the possibility that a steeper angle in the present study may have contributed to the variance in ETP.

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 >80%, except for Δ ETPLat R1 vs. R2 and R3. These latter comparisons should be viewed with caution.

Mandibular Advancement and ETP

Mandibular advancement was associated with decreases in ETP at all measured locations. This is similar to our previous findings where ETP was measured in rabbits from a single level, approximately equivalent to R2 in the current study, at anterior and lateral pharyngeal submucosal positions (14). However, unlike the present study where the magnitude of the decrease in ETP was site dependent [i.e., more pronounced at rostral tissues regions (R1 > R2 > R3) and unevenly distributed around the upper airway (ETP Lat/AntLat > Ant at R1/R2 and Lat/AntLat < Ant at R3)], the ETP change in our earlier study was not dependent on measurement site (i.e., ETPAnt = ETPLat) (14). This is likely a product of differences in experimental conditions between both studies. Previously, the upper airway was intact and conducting airflow; thus respiratoryrelated upper airway muscle activity triggered by pharyngeal negative pressure reflexes is likely to have been present (14), in addition to respiratory/lung volume-associated tracheal traction changes (15, 30). Since both muscle activity and tracheal traction alter ETP (2, 13, 16), the absence of both these influences in the present study is likely to have contributed to an altered ETP response.

Mandibular Advancement and Upper Airway Lumen Geometry

The present study demonstrated that application of mandibular advancement enlarged the upper airway lumen nonuniformly along its length, with greatest changes occurring at the hyoid region (R2) for all lumen size measures (CSA, APD, LD). Previous studies in humans have similarly shown region-



Fig. 4. Upper airway lumen reconstructions from a representative rabbit for applied levels of mandibular advancement (MA; 0-4.6mm). Note enlargement of the upper airway lumen and small decrease in upper airway length with increasing mandibular advancement. Absolute volume (Vol) of total airway lumen is indicated below each corresponding reconstruction.

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dependent increases in pharyngeal size with mandibular advancement (11, 12, 18, 23, 26, 32). In these human studies, the hypopharynx (approximately equivalent to R3 in the present study) was generally the region least influenced by mandibular advancement, which also tended to be the case in the present study. Most authors agree that in awake healthy and OSA subjects, velopharyngeal dimensions increase with mandibular advancement, but there is disagreement as to whether oropharyngeal dimensions are affected (11, 12, 18, 23, 26, 28, 29, 32). In anesthetized healthy and OSA subjects, mandibular advancement increases both velopharyngeal and oropharyngeal size, with enlargement of the oropharynx generally greater than or equal to that of the velopharynx (11, 12, 18, 19). In contrast, in anesthetized obese non-OSA subjects there is enlargement of the oropharynx but not the velopharynx (12). The present findings are most similar to those obtained in anesthetized healthy and OSA subjects, likely reflecting similar neuromechanical conditions. Similar to findings in the present study, mandibular advancement-associated anteroposterior and lateral diameter increases have also been reported in anesthetized human subjects (healthy and OSA) at velopharyngeal and oropharyngeal levels (11, 12, 18, 19), whereas in awake OSA subjects only lateral dimension increases with no anteroposterior diameter change are generally described (5, 23, 26, 33). Thus these reports suggest that mandibular advancement effects on regional pharyngeal lumen dimensions (particularly the oropharynx) appear to be influenced by the mechanical properties of the peripharyngeal tissue mass itself, in particular the presence or absence of upper airway muscle tone.

Mandibular advancement decreased upper airway length in the present study. This is likely a result of the cranial-based displacement of the hyoid bone elevating the thyroid cartilage, and hence, via thyrohyoid attachments, the base of the epiglottis (see below). To our knowledge, there are only two published studies that address the effects of mandibular advancement on upper airway length [measured from MR images (5, 26)]. Both studies were conducted in awake humans and report conflicting outcomes. One study demonstrated no length change (5), while the other reported a decrease in length (26). This discrepancy may relate to subject groups (e.g., level of obesity), degree of upper airway muscle tone, and the absolute lung volume level [i.e., amount of tracheal displacement (2)] at which measurements were made. Another probable cause is measurement error associated with image slice thickness, since both studies used the number of axial slices (multiplied by slice thickness) between defined cranial and caudal upper airway boundaries to obtain a measure of length (5, 26). In this case, the length change may not be captured or artificially measured if the change in length is less than the axial slice thickness (26). Additional studies in humans are



Fig. 6. Individual rabbit data (different symbols) and linear mixed-effects model (solid line) showing mean regional percent change in cross-sectional area (Δ CSA) from baseline with mandibular advancement (dotted line = 0%). CSA significantly increased at all upper airway regions (*A*–*C*; *P* < 0.001 for model slopes), with the greatest values for Δ CSA occurring at R2 (i.e., R2 > R1 = R3).

Table 4.	Thyroid	cartilage	and hyoid	bone	displacements
per millin	neter of	mandibul	ar advance	ment	

	Thyroid Cartilage Displacement	Hyoid Bone Displacement
X (AP), mm/mm	0.05 (0.01-0.09)	0.13 (0.07-0.18)
Y (CC), mm/mm	0.14 (0.10-0.18)	0.38 (0.32-0.44)
Z (Lat), mm/mm	NS	NS
XYZ (resultant)		
Magnitude, mm/mm	0.16 (0.12-0.21)	0.42 (0.36-0.47)
Angle, °	0 ± 2	18 ± 4

Values are linear-mixed effects model slopes, with 95% CI in parentheses, except for angle, which is mean \pm SD. *X*, *Y*, *Z*-axes are anteroposterior (AP), cranial-caudal (CC), and lateral (Lat), respectively. Displacement component directions: +*X*, anterior; +*Y*, cranial; +*Z*, lateral right; *XYZ*, resultant displacement vector, with angle relative to +*Y*-axis, within the *XY* (mid-sagittal) plane. NS, not significant. All values *P* < 0.001.

required to better define upper airway length changes associated with mandibular advancement and to establish resultant impacts on upper airway mechanics (20).

Mandibular Advancement, Peripharyngeal Tissue Mechanics, and Hyoid Bone Movement

Improvements in upper airway function and collapsibility with mandibular advancement (9, 10, 12, 17, 21) have been attributed to increases in upper airway lumen size and/or improvements in peripharyngeal tissue mechanics [e.g., tissue stiffness (11, 12)]. While increases in lumen size have been demonstrated (in this study and others), the influence of mandibular advancement on peripharyngeal tissue mechanics has received little attention. In the present study, in the absence of an increase in hydrostatic pressure in the tissues, changes in ETP are indicative of changes in mean tissue stress arising from tissue deformation (2). As such, outcomes of the present study suggest that mandibular advancement nonuniformly deforms peripharyngeal tissues, particularly at the tongue region (R1) and lateral/anterolateral tissue areas at tongue and hyoid regions (R1/R2). Furthermore, a change in ETP is also likely suggestive of changes in tissue stiffness, which would contribute to alterations in upper airway compliance with mandibular advancement (12). The mechanisms precipitating ETP and upper airway lumen geometry changes are likely to involve a number of complex interactions within the peripharyngeal tissues, including those associated with displacement of the hyoid bone.

The hyoid bone serves as a pivot point for half of the upper airway's muscles, as well as other soft tissue connections. As such, its position is likely to affect attached muscle angles, length/tension relationships, and, consequently, resultant dilatory force vectors applied to the pharyngeal airway. Furthermore, the hyoid bone is capable of transferring applied loads throughout the peripharyngeal tissues by way of its movement in response to such loads. We have previously suggested that the hyoid bone may play a pivotal role in redistributing caudal tracheal displacement (increasing lung volume) loads (2). Caudal tracheal displacement displaces the hyoid bone caudally (via thyrohyoid attachments), which allows this intervention to influence both rostral and midtissue regions (via loading of suprahyoid connections). In the present study, mandibular advancement displaced the hyoid bone in a cranial-anterior direction. This finding is similar to that generally demonstrated in humans (3, 5, 18, 26, 28). The cranial-anterior displacement of the hyoid bone with mandibular advancement likely contributes to an increase in patency and stability of the upper airway. This concept is supported by findings in anesthetized dog (31) and human cadaver studies (22) demonstrating improvements in upper airway patency with direct displacement of the hyoid bone (i.e., hyoid traction) in anterior (22, 31) and cranial-anterior directions (22). A study in awake OSA subjects also provides evidence that larger increments in hyoid bone displacement with mandibular advancement are linked to greater improvements in upper airway lumen size (3). In addition, baseline position of the hyoid bone is thought to be a contributing predictor to mandibular advancement therapy effectiveness for OSA, although reports are varied as to whether a more cranially or caudally located hyoid bone leads to improved treatment success (6, 7, 24, 27).

A schematic of potential hyoid bone, peripharyngeal tissue, and upper airway interactions with mandibular advancement, demonstrating their potential contribution to pharyngeal geometry and peripharyngeal tissue pressure outcomes in the present study, are depicted in Fig. 8. In this schema, mandibular advancement moves the hyoid bone in the direction of the applied load (i.e., cranial-anterior, as in the present study), most likely as a result of stretching mandible-originating suprahyoid muscles (geniohyoid/mylohyoid; note digastric does not interact with the rabbit hyoid), and to a lesser extent the genioglossus (transverse fibers). This hyoid movement in turn stretches infrahyoid tissue connections (e.g., sternohyoid/thy-



Fig. 7. Individual rabbit data (symbols) and linear-mixed effects model (solid line) showing the effect of mandibular advancement on displacement of the hyoid bone (dotted line = 0 mm) in anterior-posterior (X) axis (A) and cranial-caudal (Y) axis (B). Arrows indicate direction of displacement. Mandibular advancement results in movement of the hyoid bone in both anterior and cranial directions (P < 0.001 for model slopes).

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Fig. 8. A schematic (lateral view) of the rabbit upper airway (UA) showing selected muscles (*A*; thick black lines) and the potential role of the hyoid bone (and tongue) in transferring mandibular advancement (MA) loads to areas throughout the UA (*B*; white arrows indicate direction of muscle stretch; dashed structures indicate preload condition). When MA is applied (*B*), the geniohyoid and mylohyoid are directly loaded and stretched, displacing the hyoid bone in a cranial-anterior direction. This movement likely transfers the MA load to caudal-region anterior tissues [thyrohyoid attachments (i.e., thyrohyoid muscle, ligaments and connective tissue; that also lift the thyroid cartilage), sternohyoid and omohyoid (not shown) muscles] and midregion lateral tissues [middle constrictor (Middle Const.) and stylohyoid (SH) muscles]. This potentially stiffens the UA tissue wall in these areas and enlarges the airway lumen laterally (not shown in diagram) through the lateral tissue connections. In addition, MA leads to displacement of the tongue which likely pulls on the soft palate via the palatoglossus (PG) to enlarge the airway in this region and stiffen/enlarge the airway laterally [e.g., via the styloglossus (SG) muscle]. Tongue movement may also assist in displacement of the hyoid via the hyoglossus (HG) and genioglossus (transverse fibers).

rohyoid muscles), reflected in the present study by the decrease in ETP at the epiglottis region. Stretch of infrahyoid connections subsequently displaces the thyroid cartilage in a primarily cranial direction (as also shown in the present study), likely stiffening anterior tissues in this region. Thus, in this manner, the hyoid bone operates as a pivot transferring the rostral region-based mandibular advancement load to the caudal peripharyngeal tissues. Furthermore, displacement of the hyoid bone likely stretches its lateral tissue connections (middle constrictor and stylohyoid muscles), which, alongside tissue changes associated with movement of the tongue (4), may enlarge the airway lumen laterally (particularly via stretch of the middle constrictor) and stiffen the lateral pharyngeal walls. In support of this concept, anterolateral and lateral tissue pressure decreases were greatest in the present study at the rostral and midtissue regions where the stylohyoid and middle constrictor muscles are located. A consequence of this conceptual analysis is that the ability of mandibular advancement to enlarge or stiffen the upper airway may be impaired if hyoid bone movement is restricted or hyoid bone geometrical relationships (e.g., abnormally positioned hyoid bone phenotype) work to reduce the effectiveness of dilatory force vector transmission to upper airway walls.

In addition to peripharyngeal tissue changes associated with hyoid bone displacement, mandibular advancement likely influences several other muscles that form the lateral/anterolateral walls of tongue and hyoid regions (R1/R2). The mandibleattached digastric and superior constrictor muscles are likely directly stretched, alongside forward movement of the tongue. Tongue movement also likely influences the superior constrictor muscle (via its tongue attachments), in addition to other tongue-inserting muscles such as the styloglossus and palatoglossus (Fig. 8). A previous hypothesis suggested that improvements in velopharyngeal collapsibility are partially a result of palatoglossus muscle stiffening (11, 12), which occupies part of the lateral/anterolateral tissue areas at the tongue region in the present study, where decreases in tissue pressure were largest. Indeed this muscle is also likely to contribute to improvements in upper airway size in this region as it pulls down on the soft palate, particularly increasing anteroposterior dimensions (Fig. 8), as observed in the present study.

In summary, this study has shown that mandibular advancement results in nonuniform decreases in peripharyngeal tissue pressure (i.e., increases in hydrostatic stress) in conjunction with nonuniform increases in upper airway lumen size. The present study's findings suggest that movement of the hyoid bone may play an important role in redistributing the mandibular advancement load throughout the peripharyngeal tissue mass. We speculate that phenotypic differences in hyoid bone mechanics between patients may explain, in part, the variable success of mandibular advancement as a therapy for OSA.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

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