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Hyolaryngeal kinematics and swallow patterning in normal and disordered swallowing

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Hyoaryngeal Kinematics and Swallow Patterning In Normal and Disordered Swallowing

Seng Mun Wong

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Abstract

Hyoid and laryngeal movements contribute to laryngeal vestibule closure and upper esophageal sphincter (UES) opening for safe swallowing. However, the extent of movement required for achieving these goals, and the interaction between hyoid and laryngeal movements during swallowing are unknown. Despite impairment in vestibule closure and UES opening, patients with dysphagia may exhibit reduced, increased or similar hyolaryngeal displacements as healthy individuals. This limits the delineation between normal and disordered swallowing. We investigated whether anatomical differences in hyolaryngeal positions and the extent of laryngeal vestibule opening at rest would better predict hyolaryngeal displacements and the extent of vestibule closure during swallowing than neck length. We then examined if hyolaryngeal maximal displacements that corrected for individual anatomical differences would show greater contrast between the swallows of patients and healthy individuals than uncorrected measures. We also investigated if the relationship between hyoid and laryngeal elevation, as well as measures of laryngeal elevation peak velocity, timing and movement patterning would differ between patients and controls swallowing more than corresponding measures of hyoid elevation.

Videofluoroscopic examinations of swallowing were performed in healthy adults and patients with dysphagia. Using frame-by-frame motion analysis, measures of forward and upward hyolaryngeal displacements and velocities, and vestibule area were made during swallowing. In healthy volunteers, the extent of laryngeal vestibule opening at rest predicted the extent to which laryngeal elevation exceeded hyoid elevation for closing the space between the hyoid and larynx during swallowing. Spatially normalized measures of hyoid and laryngeal elevation magnitudes showed greater differences between normal and abnormal swallowing than raw measures. Patients with dysphagia had insufficient laryngeal elevation relative to hyoid elevation to achieve vestibule closure during swallowing. In conclusion, healthy individuals may adapt hyolaryngeal movement magnitudes according to changes in the movement targets required for vestibule
closure to ensure safe swallowing. Insufficient laryngeal elevation relative to hyoid elevation may be detrimental to airway protection for swallowing in dysphagia.
Introduction

Swallowing or deglutition is an integral part of eating and drinking. Its underlying neural control is complex. Swallowing is understood to be a centrally patterned response involving motor pattern generation in the brainstem when sensory input exceeds activation threshold (Jean, 2001; Jean & Car, 1979; Kessler & Jean, 1991; Miller, 1972). The cortex is also highly influenced by sensory feedback (Lowell et al., 2008; Martin, Murray, Kemppainen, Masuda, & Sessle, 1997; Murray & Sessle, 1992; Soros et al., 2008), and exerts volitional control over the brainstem by modulating the onset and magnitude of the swallow response (Martin, Goodyear, Gati, & Menon, 2001; Martin et al., 1999; Martin et al., 1997). The motor response for swallowing culminates as synchronized movements of the oral and pharyngeal structures. These coordinated movements ensure safe and timely transport of food or liquid in the form of a bolus from the oral cavity into the esophagus.

Movements of the hyoid bone and the larynx during swallowing are thought to protect the airway from bolus entry and to open the upper esophageal sphincter (UES) for bolus entry into the esophagus. Superior (upward) and anterior (forward) laryngeal movements may protect the airway by shortening the distance between the hyoid bone and the larynx, and allowing the epiglottis to fold over the ascending larynx (Ekberg & Sigurjonsso, 1982; Logemann et al., 1992).

The UES is thought to be tonic at rest due to central inhibitory neural firing, but relaxes when inhibitory inputs cease during swallowing as part of the patterned response from the brainstem (Zoungrana, Amri, Car, & Roman, 1997). Kinematic analysis and manometry have shown that UES relaxation precedes sphincter opening (Cook et al., 1989; Williams, Pal, Brasseur, & Cook, 2001). Sequential hyoid and laryngeal movements in both superior and anterior directions may influence UES opening. Superior hyoid and laryngeal excursion are thought to occur first (Cook et al., 1989; Williams et al., 2001), followed by rapid anterior hyoid movement that pulls the larynx forward and exerts traction to open the anterior aspect of the UES.
(Cook et al., 1989; Jacob, Kahrilas, Logemann, Shah, & Ha, 1989; Williams et al., 2001; Yokoyama, Mitomi, Tetsuka, Tayama, & Niimi, 2000). Superior laryngeal elevation may aid posterior UES opening by stretching the posterior sphincter wall (Jacob et al., 1989). Thus superior and anterior hyoid movements and superior laryngeal excursion appear crucial in UES opening, whereas anterior laryngeal motion may be passive and consequential to traction by anterior hyoid movement (Kahrilas, Logemann, Krugler, & Flanagan, 1991). The success of UES opening may depend highly on the timely coordination of these hyolaryngeal movements along with reflexive UES relaxation. Superior laryngeal movement may begin relatively early in the pharyngeal swallow due to its involvement in initiating upper airway protection and UES opening.

Kinematic measures of swallowing aim to quantify the movement characteristics of structures involved in swallowing, separate from the forces that produce the movements (Sutherland, 1997). These measures include hyoid and laryngeal movement magnitudes during swallowing (Molfenter & Steele, 2011), their movement velocities (Kahrilas, Lin, Rademaker, & Logemann, 1997; S. H. Lee et al., 2013; Nagy, Molfenter, Peladeau-Pigeon, Stokely, & Steele, 2014; Prosielgel, Heintze, Sonntag, Schenk, & Yassouridis, 2000) and the durations and intervals between swallowing events (Kahrilas et al., 1997; Lee, Yoo, Kim, & Ryu, 2013; Molfenter & Steele, 2012). Duration has been defined as the time taken for a particular motor act, such as the duration of UES opening (Molfenter & Steele, 2012). Interval refers to the time taken between swallowing events, for example, the interval between bolus entry into the pharynx and bolus passage through the UES (Molfenter & Steele, 2012). Displacement and velocity measures reflect the amplitude and speed of structure movement in the pharyngeal swallow, while duration and interval measures may reflect motor coordination associated with the swallow pattern.

Systematic reviews have identified large inter-subject variability within the normal population in swallowing duration and interval measures, and in hyolaryngeal displacement
Variability may arise from: methodological differences across studies; failure to account for individual differences in size; age-related changes in the swallowing mechanism; and, inherent population variance (Molfenter & Steele, 2011, 2012). Variability among individuals without swallowing impairment or dysphagia may mask the ability to quantify differences between normal and disordered swallowing. For instance, superior hyoid displacement during 10 ml liquid swallows in healthy males can vary as much as 7 mm to 18 mm (Ishida, Palmer, & Hiiemae, 2002; Logemann et al., 2000). Patients with dysphagia post-stroke were found to have superior hyoid displacements within the normal range between 14 mm and 16 mm (Y. Kim & McCullough, 2010). When patients and controls were compared within a study, anterior hyoid displacement did not differ between stroke patients and healthy controls (Bingjie, Tong, Xinting, Jianmin, & Guijun, 2010; Paik et al., 2008). Similarly, anterior-superior laryngeal excursion did not vary between controls and dysphagic patients (Sundgren, Maly, & Gullberg, 1993).

As patients with dysphagia often exhibit reduced airway protection and limited opening of the UES, we would expect hyoid and laryngeal excursions to be reduced in disordered swallowing. However, the above findings contradict this. Furthermore, patients with Parkinson’s disease were found to have greater superior hyoid, anterior laryngeal and superior laryngeal excursions than controls (Y. H. Kim et al., 2014), which was also contrary to expectation. Hyoid displacement magnitudes have not consistently improved with dysphagia intervention (van der Kruis, Bajens, Speyer, & Zwijnenberg, 2011). These results suggest that hyoid and laryngeal displacement measures cannot determine the severity of swallowing impairment or quantify change in swallowing function. Outcome measures sensitive to treatment benefits and motor recovery or compensation are needed (Levin, Kleim, & Wolf, 2009).

Reducing variability in hyoid and laryngeal displacement measures in normal swallowing may be the first step in improving discrimination between healthy controls and dysphagic patients. In locomotion research, normalizing walking stride length by the individual’s leg length
may reduce inter-subject variability by 54% compared to measuring stride length in raw units (Pierrynowski & Galea, 2001). Other researchers also advocate correcting for individual differences in anatomical size to increase differentiation between groups (Carty & Bennett, 2009; Hof, 1996; Stansfield et al., 2003).

In swallowing, correcting for individual differences in head and neck anatomy may reduce variability in hyolaryngeal displacement measures. Anatomical differences proposed for correction include: distance between the 2nd (C2) and 4th (C4) cervical vertebrae (Kang et al., 2010; Logemann et al., 2000; Logemann, Pauloski, Rademaker, & Kahrilas, 2002; Molfenter & Steele, 2011, 2014; Nagy et al., 2014; Steele et al., 2011); distance between C1 and C5 (Perlman, VanDaele, & Otterbacher, 1995); length of the mandible (Kang et al., 2010); the horizontal distance between the mandible and the hyoid (Kahrilas et al., 1997); distance between the arytenoids and the valleculae (Kahrilas et al., 1997); and, the Frankfort-mandibular-plane angle (Mays, Palmer, & Kuhlemeier, 2009).

Correcting for differences in neck length using C2 to C4 length is the most common approach. This method removes male and female differences in hyoid displacement during swallowing (Molfenter & Steele, 2014), and increases differences in hyoid excursion between young and old individuals (Kang et al., 2010; Logemann et al., 2000). Perlman et al. (1995) found a larger mean difference between 2 patient groups when superior hyoid displacement was expressed as a percentage of C1 to C5 length rather than in raw units (mm). However, hyoid and laryngeal displacements normalized by C2 to C4 length failed to distinguish patients with bolus penetration into the airway from those without (Steele et al., 2011). Thus the ability of displacement measures normalized by neck length to differentiate patient groups is inconclusive. Furthermore, although accounting for neck length may reduce sex differences due to size and enhance age or group differences, it does not explain how variation in neck length across people might alter hyoid and laryngeal kinematics, upper airway protection and UES opening. As the hyoid bone is suspended by suprathyroid and infrahyoid muscles that are not attached to the
cervical spine, some have proposed that neck or spine length may relate to the length of the pharyngeal cavity in which swallowing movements occur and thus influence the extent of hyolaryngeal movement (Molfenter & Steele, 2014).

During development the relative distances between the hard palate, mandible, hyoid and larynx remain constant from infancy to adolescence despite hyolaryngeal descent and changes in vertical and horizontal orofacial dimensions (Lieberman, McCarthy, Hiimeae, & Palmer, 2001). Maintenance of these spatial relationships may be crucial for preserving swallow movement patterning, so that continual adaptation to orofacial growth is minimized, and swallowing function can remain safe and efficient throughout early development (Lieberman et al., 2001). The distances between structures in the pharyngeal and laryngeal regions may have biomechanical and functional relevance to the swallowing motor pattern. These distances may be more important than neck or pharyngeal length in determining the extent of hyoid and laryngeal movements required for safe swallowing.

Speech motor control may overlap with swallowing control at the cortical, brainstem, peripheral and structural levels (McFarland & Tremblay, 2006). Movement magnitudes of the articulators appear to depend on the extent of movement needed to achieve the functional goals for intelligible speech, rather than the overall size of the articulatory system (Riely & Smith, 2003). Thus jaw displacement magnitude did not vary between children and adults during speech production despite obvious differences in facial size (Riely & Smith, 2003). In another example, perturbation that widened inter-labial distance during bilabial sounds increased lip movement, demonstrating that movement magnitude may be scaled according to the displacement required to fulfill the functional goal of bilabial contact (Gracco & Abbs, 1985). Translating these findings to swallowing, the magnitudes of hyoid and laryngeal displacements during swallowing might be scaled according to the extent of movement required to achieve the functional goals of airway protection and UES opening, rather than based on the overall length.
of the pharynx or the neck. The first 2 manuscripts reported how this hypothesis was tested, and determined if displacement measures normalized by length or size required for safe swallowing will differentiate normal and disordered swallowing better than raw measures.

Displacement measures alone do not fully characterize the swallowing motor response. To determine the integrity of movement patterning and coordination, both spatial and temporal measures should be considered (Krasovsky & Levin, 2010). For hyoid and laryngeal movements, the time when movement begins, when the most rapid movement occurs and when the peak displacement occurs seem to differ between normal and disordered swallowing (Bisch, Logemann, Rademaker, Kahrilas, & Lazarus, 1994; Kahrilas et al., 1997; Kendall & Leonard, 2001; Y. Kim & McCullough, 2010; Y. H. Kim et al., 2014; Power et al., 2007). This may suggest an overall slower swallowing motor response in disordered swallows. For example, Kahrilas et al. (1997) found that delayed laryngeal vestibule closure and UES opening relative to the onset of glossopalatal separation correlated with the severity of laryngeal penetration. They proposed that delayed vestibule closure may be related to late onset and reduced velocity of superior laryngeal elevation, while delayed UES opening may be associated with delayed onset and reduced velocity of anterior hyoid movement. Similarly, delayed onset of anterior-superior hyoid displacement relative to the time of bolus entry into the oropharynx may distinguish between stroke patients with aspiration and those without (Y. Kim & McCullough, 2007), and between patients and healthy controls (Kendall & Leonard, 2001). Power et al. (2007) and Bisch et al. (1994) also found that the onset of superior laryngeal displacement relative to the time of bolus entry into the pharynx occurred later in patients than in healthy subjects, and this correlated with penetration/aspiration severity (Power et al., 2007). Others have found that the occurrence of rapid hyoid movement (time of peak velocity) may be highly correlated with the time at which laryngeal vestibule closure occurs when both measures are expressed as percentages of the time taken for the hyoid to travel from rest to its peak position, suggesting a functional significance for measuring the time of peak velocity (Nagy et al., 2014). However,
they did not investigate if the time of rapid laryngeal movement was also correlated with the time of laryngeal vestibule closure. When the time at which peak hyoid and laryngeal displacements occur was measured relative to bolus entry into the pharynx, patients with Parkinson’s disease showed a delay compared to age-matched controls that concurred with bradykinesia associated with the disease (Y. H. Kim et al., 2014).

Measuring the occurrence of events in s or ms relative to bolus arrival in the pharynx, as reported in these studies, examine if the motor response is slow or delayed, but does not inform about the pattern or coordination of movement. Measures of mean and peak velocities also may not quantify motor patterning (Krasovsky & Levin, 2010). Thus swallows that exhibit overall slow movement cannot be distinguished from swallows with deviant movement patterns using absolute time measures. Furthermore, temporal measures in s or ms are found to vary widely in normal swallowing just like displacement measures in cm or mm (Molfenter & Steele, 2012). For example, a meta-analysis demonstrated that the interval between bolus entry into the pharynx and the onset of hyoid movement in healthy subjects varied between -0.22 and 0.54 s across studies (Molfenter & Steele, 2012). However, the same measure from patients also fell within this range (Bisch et al., 1994; Y. Kim & McCullough, 2007; Power et al., 2007). In view of this, some researchers have taken a more gestalt approach by examining overall swallow patterning in velocity against time plots. This is based on the observation that normal movement in one direction has a single peak in the plot of velocity against time (Flash & Hogan, 1985).

When the hyoid or larynx moves forward and backward or upward and downward during swallowing, the corresponding velocity over time curve will have a positive peak (e.g. during most rapid laryngeal elevation) and then a negative peak when the direction of movement changes (e.g. when the larynx descends most rapidly). Multiple velocity peaks during motion may characterize impaired movement coordination or reduced movement smoothness (Cirstea & Levin, 2000; Rohrer et al., 2002). The recovery of motor function may be associated with the
gradual reduction of these extraneous peaks to one velocity peak per movement per direction (Rohrer et al., 2002).

In swallowing, velocity curves over time appear to show differences between patients and controls. Hyoid and laryngeal velocity curves showed multiple peaks in patients with neurogenic dysphagia (Y. H. Kim et al., 2014; Paik et al., 2008; Prosiegel et al., 2000). Patients with myopathy appeared to have swallow patterning similar to healthy controls despite having reduced hyoid displacement and velocity, suggesting that peripheral neuropathy did not impair central swallow patterning (Paik et al., 2008). Conversely, stroke patients had deviant swallow patterning with multiple hyoid velocity peaks while hyoid displacement magnitudes did not differ from healthy controls (Paik et al., 2008). These findings indicate that swallow patterning, slowness in movement and displacement magnitude may distinguish patients from controls in different ways. Movement patterning, timing delays and movement magnitudes may be crucial in delineating dysphagia of different etiologies. Further, some measures may differentiate improvement in function due to central neural recovery of the original substrate, from that due to compensation (Levin et al., 2009).

Time normalization may reduce variability when analyzing movement patterning (Helwig, Hong, Hsiao-Wecksler, & Polk, 2011; Smith, Goffman, Zelaznik, Ying, & McGillem, 1995). The utility of time-normalized temporal measures in discriminating between normal and disordered swallowing has been explored subjectively but not quantitatively. Paik et al. (2008) defined the swallowing cycle as the interval between the onset of superior hyoid movement to its return to resting position. They compared hyoid movement patterns between normal and disordered swallowing using time-normalized trajectory plots, but did not quantitatively examine differences in normalized timing of events between patients and controls. In contrast, locomotion and motor speech research have utilized normalized timing of events to investigate whether an invariant motor pattern underlies movement in different conditions (Shapiro, Zernicke, & Gregor, 1981; Smith et al., 1995).
As the success of UES relaxation and opening in swallowing may be highly dependent on timely coordination among anterior and superior hyoid and laryngeal movements, the failure of bolus passage through the UES may be indicative of a swallow patterning issue rather than slowness in movement. Thus time-normalized measures may characterize this type of swallow impairment. The 3rd manuscript reported findings on whether patients and healthy volunteers could be differentiated in hyolaryngeal movement velocities, patterning and coordination using temporal measures of swallowing, and whether patient swallows with vs. without UES opening could be differed in these measures.
TITLE
Predicting hyolaryngeal movement during normal swallowing

RUNNING HEAD
Predicting hyolaryngeal movement
ABSTRACT

Hyoid and laryngeal displacements contribute to laryngeal vestibule closure and upper esophageal sphincter (UES) opening during swallowing. However, the extent of hyolaryngeal movement required to achieve these functional goals is unknown, except that neck length may predict hyoid elevation magnitude during swallowing. Stride length during walking may be scaled by body size, but in speech, the movement distance required to reach the articulatory target for intelligible speech may better predict articulatory movement magnitude than facial size. Swallowing may be similar to speech production in that hyolaryngeal displacement magnitudes may be scaled by the extent of movement required for vestibule closure and UES opening. We investigated whether hyolaryngeal positions and the extent of laryngeal vestibule opening at rest would better predict hyolaryngeal displacements and the extent of vestibule closure during swallowing than neck length. We also investigated if changes in head position would alter hyolaryngeal positions and the extent of laryngeal vestibule opening at rest, and whether individuals would then adapt by adjusting hyolaryngeal movement magnitudes during swallowing. Videofluoroscopy was performed in 26 healthy adults. Using frame-by-frame motion analysis, maximum forward and upward hyolaryngeal displacements and vestibule area during swallowing were measured. These were correlated with neck length, hyolaryngeal positions and extent of vestibule opening at rest. The extent of vestibule opening at rest predicted the extent to which laryngeal elevation exceeded hyoid elevation for closing the space between the hyoid and larynx during swallowing. Anterior laryngeal displacement was predicted by larynx position at rest. Hyoid elevation was predicted by neck length and hyoid position at rest. No significant predictors of anterior hyoid displacement were found. Individuals may adapt hyolaryngeal movement magnitudes according to changes in the movement targets required for vestibule closure to ensure safe swallowing.
KEYWORDS

Deglutition, hyoid, larynx, vestibule, spatial normalization, adaptation
INTRODUCTION

Swallowing or deglutition is understood to be a centrally controlled motor pattern generated in the brainstem when sensory input exceeds the threshold for activation (16, 17, 22, 33). The cortex is also highly influenced by sensory feedback (28, 31, 37, 45) and exerts volitional control over the brainstem by modulating the onset and magnitude of the swallow response (29-31). The swallow motor response culminates as synchronized movements of the oral and pharyngeal structures for safe and timely bolus transport from the oral cavity into the esophagus.

Laryngeal vestibule closure and upper esophageal sphincter (UES) opening are requisites for safe swallowing, and are thought to be contributed by upward and forward displacements of the hyoid and the larynx (4, 6, 7, 14, 19, 27, 51, 52). Factors that determine the extent of hyolaryngeal displacements needed during swallowing in order to close the vestibule and open the UES are not fully known. Some studies found a correlation between the extent of hyoid elevation during swallowing and the length of the neck between the second (C2) and fourth cervical vertebrae (C4), which may in turn vary according to the height of the individual (20, 35). Maximum anterior hyoid excursion during swallowing may be related to the distance between the mandible and the cervical spine (20), and the inclination of the lower face relative to the cranium (32). Anatomical factors that predict the extent of anterior and superior laryngeal movement during swallowing are unknown, except that an individual’s height does not seem to predict the extent of laryngeal approximation to the hyoid during swallowing (25).

Gross anatomy of the neck and face might have some contribution to movement magnitudes in swallowing, as 14 % and 50 % of the variance in hyoid displacement may be explained by neck length and facial structure respectively (32, 35). This is analogous to how leg length might explain differences in stride length among individuals during walking (3, 10, 46). However, these measures of gross head anatomy may not have direct functional relevance to the
goals of vestibule closure and UES opening in swallowing. They also do not explain how hyolaryngeal movement magnitudes may change in different swallowing conditions while neck length and facial structure stay the same, for example, when swallowing in supine position (40) or in a chin-tuck posture (23, 50). In the production of skilled movements such as speech, jaw displacements were found to be similar between children and adults, and among adults of varying orofacial sizes (42). When the distance between the lower and upper lip during bilabial production was abruptly increased by perturbation lowering the jaw, individuals adapted by increasing lip displacement to achieve bilabial closure (9). These findings suggest that articulators may vary in their movement magnitudes dependent upon the movement distance required to achieve a functional goal required for intelligible speech (9). Swallowing is a form of skilled movement with vestibule closure and UES opening being two important goals for safe swallowing. Therefore, hyolaryngeal displacement magnitudes may be scaled by the movement distances required to approximate the larynx to the hyoid to close the vestibule, and to displace the hyolaryngeal complex forward for UES opening. Spatial relationships among the mandible, hyoid and larynx were found to be consistent between infancy and adolescence despite hyolaryngeal descent and orofacial growth (26). This may ensure accurate movement patterning for safe and efficient swallowing as the individual adapts to orofacial growth throughout early development (26).

Based on evidence in speech motor control and orofacial development, we hypothesized that anatomical measures of the distances and areas among the hyoid, larynx, mandible and the cervical spine may have greater biomechanical and functional relevance to the execution of patterned swallowing movements for vestibule closure and UES opening. We investigated this in 2 studies. In Study 1, we examined the relationships between anatomical measures at rest, and hyolaryngeal movement magnitudes and the extent of vestibule closure between the hyoid and the larynx during swallowing in a group of healthy individuals. We hypothesized that
anatomical measures of the distances and areas among the hyoid, larynx, mandible and the cervical spine at rest would better predict the extent of hyoid and laryngeal movements required for safe swallowing than neck length. In Study 2, we manipulated hyolaryngeal spatial configurations at rest by asking another group of healthy individuals to swallow in different head positions relative to a neutral, comfortable position, and then measured corresponding changes in hyolaryngeal maximal displacement magnitudes and vestibule closure during swallowing. We hypothesized that in healthy individuals, changes in head position would alter the spatial relationships among the hyoid, larynx, mandible and the cervical spine, and that individuals would adapt to these changes by altering hyolaryngeal movement magnitudes to maintain vestibule closure and UES opening.

STUDY 1

MATERIALS and METHODS

Subjects

Healthy adults between 20 and 80 years old were recruited as volunteers, and gave informed consent to participate in protocols approved by the Institutional Review Boards (IRBs) at James Madison University and Sentara Rockingham Memorial Hospital Medical Center. Volunteers who reported the following were excluded based on screening questionnaire: swallowing difficulty, history of neurological disorder affecting swallowing function, acid reflux diagnosed by a physician, and history of head and neck cancer. De-identified archived video recordings of healthy volunteers gathered under IRB approved archival protocols from the National Institute of Neurological Disorders and Stroke were also used.
Procedure

A radio-opaque ball with a 19 mm diameter was taped to the side of the subject’s neck posterior to the spine to be used for measurement calibration of pixels into millimeters. A digital Siemens fluoroscope (Model AXIOM Luminos TF) was set up for a lateral view from anterior neck extending inferiorly from the trachea and below the upper esophageal sphincter, to posterior spine from C1 to C6 and extending superiorly to the floor of the nasal cavity (Fig. 1). A syringe containing 5 ml of thin liquid barium (Varibar®, 40% weight-volume) was delivered orally by the examiner. The fluoroscope was then turned on and the examiner instructed the participant to “swallow now”. Magnification was unchanged throughout the swallow. Each fluoroscopic swallow trial was captured at 30 frames/s and saved in .avi format using a D-scope® System (D-scope® Systems, Brooklyn, NY).

Data processing

Recordings were imported into Peak Motus 8.5 (Vicon Denver, Centennial, CO) for distance calibration and two-dimensional motion analysis. One swallow trial per subject was analyzed.

Measure of airway protection: The videofluoroscopic recording of each swallow trial was rated on the Penetration Aspiration Scale (PAS) (43) to assess the integrity of airway protection.

Conversion into millimeters: To convert pixels into millimeters, the diameter of the calibration ball was measured on a single video frame in the video of each swallow trial. As fluoroscopic magnification did not change throughout the swallow trial, the same scaling factor was automatically applied to the other frames in the same recording. For consistency across trials, the calibration frame was when the head of the bolus reached the angle of the mandible.

Spatial analysis: The anterior-inferior corner of C4 served as the origin for the x and y-axes in the horizontal and vertical dimensions respectively. The y-axis connected the origin to the anterior-inferior corner of C2, while the x-axis was perpendicular to the y-axis at the origin (Fig.
1) A spatial model of the measurement points for motion analysis was set up in Peak Motus 8.5 to manually track the position of each point frame by frame using a cursor. Points measured on each frame of the video recording were (Fig. 2): 1) Anterior-inferior corner of C2; 2) Anterior-inferior corner of C4; 3) Anterior-inferior corner of the hyoid bone; 4) Anterior-superior corner of the subglottic air column to track the larynx; 5) Posterior-superior corner of the subglottic air column; and, 6) Posterior-inferior corner of the mandibular symphysis.

Segmental distances of interest between measurement points were also derived for each frame in a video recording. These distances were (Fig. 2): i) Distance between the anterior-inferior corner of the hyoid bone (Point 3) and the posterior-superior corner of the subglottic air column (Point 5); ii) B) Distance between the anterior-inferior corner of the hyoid bone (Point 3) and the anterior-superior corner of the subglottic air column (Point 4); iii) Distance between the anterior-superior (Point 4) and the posterior-superior corners of the subglottic air column (Point 5); iv) Horizontal distance between the anterior-inferior corner of the hyoid bone (Point 3) and the y axis; v) Vertical distance between the anterior-inferior corner of the hyoid bone (Point 3) and the horizontal line connecting the posterior-inferior corner of the mandibular symphysis (Point 6) perpendicularly to the y axis; vi) Distance between the anterior-inferior corners of C2 (Point 1) and C4 (Point 2); and vii) Horizontal distance between the anterior-superior corner of the subglottic air column (Point 4) and the y axis.

**Time periods measured:** Measurement for each swallow started on the frame that was 1s before the head of the bolus reached the angle of the mandible, and continued until 1s after the tail of the bolus passed the anterior-inferior corner of C6 (Fig. 3). However, if the hyoid and larynx had already begun movement 1 s before the bolus head reached the mandibular angle, then motion analysis began further back in time closer to the start of the fluoroscopic recording, to capture the resting positions of the hyoid and larynx while the bolus was held in the oral cavity. The rationale for using the positions prior to swallow initiation was that motor planning for hyoid and laryngeal motion is proposed to begin with oral sensory processing when the bolus is in the oral cavity (5).
Therefore this time point would capture the spatial configuration of the pharyngeal and laryngeal structures during motor planning, which may relate to subsequent airway protection and UES opening during swallowing.

**Filtering the kinematic time series data:** A fourth-order zero time lag Butterworth low-pass filter with a cutoff frequency of 4 Hz was applied within Peak Motus 8.5 to smooth the time series kinematic data for \( x \) and \( y \) over time. As recursive forward and backward passes were made in the filter process, no time lag was expected in the filtered data. The smoothed position and segmental distance time series data were exported into Matlab R2013a (The Mathworks, Inc., Natick, MA).

**Anatomical measures made at rest before swallowing onset** (Fig. 2): The following measures were derived from the first data point in the smoothed position and segmental distance time series (i.e. at least 1 s before the head of the bolus reached the mandibular angle).

A) **Distance between the anterior-inferior corner of the hyoid bone and the posterior-superior corner of the subglottic air column.** This represented the opening of the laryngeal vestibule at rest. The posterior rather than anterior corner of the subglottic air column was used, as this point represented the position of the larynx and the cricopharyngeus muscle or UES before swallowing.

B) **Area of the space between the hyoid and larynx.** This was calculated by applying Heron’s formula (48) to the triangle bound by the anterior-inferior corner of the hyoid bone, the anterior-superior corner of the subglottic air column and the posterior-superior corner of the subglottic air column. This area represented the size of the laryngeal vestibule at rest.

C) **Horizontal distance between the anterior-inferior corner of the hyoid bone and the \( y \)-axis where they intersect at 90° (i.e. the \( x \) coordinate of the anterior-inferior corner of the hyoid bone).** This was indicative of hyoid position in the anterior-posterior (AP) plane.

D) **Vertical distance between the anterior-inferior corner of the hyoid bone and the posterior-inferior corner of the mandibular symphysis.** This was indicative of hyoid position in
the superior-inferior (SI) plane, and calculated by subtracting the y coordinate of the anterior-inferior corner of the hyoid bone from the y coordinate of the posterior-inferior corner of the mandibular symphysis when both were projected onto the y axis at 90°.

E) Area of the rectangle bound by measures C and D, which represented the combined AP and SI positions of the hyoid bone before swallowing.

F) Distance between C2 and C4, which represented neck length.

G) Horizontal distance between the anterior-superior corner of the subglottic air column and the y-axis (i.e. the x coordinate of position of the anterior-superior corner of the subglottic air column). This represented larynx position in the AP plane.

Displacement measures from kinematic data: The initial positions of the hyoid and larynx were linearly transposed so that all initial positions (i.e. the first data point) had a displacement of 0 mm (Fig. 3). The following displacement measures were computed from the position time series of the hyoid and larynx.

Maximum superior laryngeal displacement (LYmax) = difference between the maximum and initial positions in the smoothed y over time series of the anterior-superior subglottic air column.

Maximum anterior laryngeal displacement (LXmax) = difference between the maximum and initial positions in the smoothed x over time series of the anterior-superior subglottic air column.

Maximum superior hyoid displacement (HYmax) = difference between the maximum and initial positions in the smoothed y over time series of the anterior-inferior corner of the hyoid bone.

Maximum anterior hyoid displacement (HXmax) = difference between the maximum and initial positions in the smoothed x over time series of the anterior-inferior corner of the hyoid bone (Fig. 3).

Maximum hyoid movement in the combined anterior and superior directions (HXY Area) = (HXmax x HYmax) / 2. This was the area of a right angle triangle bound by the maximum anterior and superior hyoid displacements.
Measure of laryngeal vestibule closure and the relationship between the larynx and hyoid during swallowing:

Difference between maximum superior laryngeal displacement and maximum superior hyoid displacement (LYHYmaxDiff) = Lymax – HYmax. This represented the relationship between maximum laryngeal and hyoid elevation during swallowing.

Minimum area between hyoid and larynx (HLarea_min) (mm²). This represented the minimum area between the hyoid and larynx during swallowing. It was derived by applying Heron’s formula (48) to the time series of the segmental distances between these 3 points: 1) anterior-inferior corner of the hyoid bone, 2) anterior-superior corner of the subglottic air column, and, 3) posterior-superior corner of the subglottic air column (Fig. 2), and then identifying the minimum value.

Statistical analyses

Measurement reliability. The same investigator replicated all of the measures for subjects for intra-rater reliability. For each of the hyoid and laryngeal displacement measures and anatomical measures, a single-measure intra-class correlation coefficient (ICC) was computed based on a two-way random effects model (assuming the effects of subject and swallow trial were random). The absolute measurement error in mm (absolute difference between the first and second measure) was also computed. The first data set was used in subsequent analyses.

Relationships between anatomical measures and displacement measures. Simple linear regressions were conducted to determine if the anatomical measures predicted maximum displacement or vestibule closure measures made for each swallow. Bonferroni-corrected alphas were used to correct for multiple predictors. For Lymax, 3 predictors were tested (α = .017): hyoid to posterior-superior subglottic air column distance, area between the hyoid and larynx, and C2 to C4 distance. For LXmax, 2 predictors were tested (α = .025): horizontal distance between the larynx and the γ-axis (spine), and C2 to C4 distance. For HYmax, 2 predictors were tested (α
vertical distance between the hyoid and the mandible, and C2 to C4 distance. For HXmax, 2 predictors were tested ($\alpha = .025$): horizontal distance between the hyoid and the $y$-axis (spine), and C2 to C4 distance. The relationship between HXY Area and area of the rectangle representing vertical and horizontal hyoid position was tested ($\alpha = .05$). The relationship between LYHYmaxDiff and hyoid to posterior-superior subglottic air column distance was tested ($\alpha = .05$). Lastly, the relationship between HLarea_min and area between the hyoid and larynx at rest was tested ($\alpha = .05$). If more than 1 anatomical measure predicted a particular displacement measure, then a multiple regression was conducted with simultaneous entry of the predictors to examine which anatomical measure(s) had significant unique contribution in predicting movement magnitude ($\alpha = .05$). Effect sizes were determined using $r^2$ values.

Relationship between laryngeal vestibule closure and hyolaryngeal displacement. We examined whether maximal hyoid and laryngeal displacements were related to the amount of vestibule closure between the hyoid and the larynx during swallowing. Simple linear regressions were conducted between reduction in vestibule area during swallowing (Area between hyoid and larynx at rest minus HLarea_min, mm$^2$) and LYmax, LXmax, HYmax, HXmax, and LYHYmaxDiff. A Bonferroni-corrected alpha of .01 was used to correct for multiple analyses.

Spatial normalization. Anatomical distance or area measures that significantly predicted hyoid or laryngeal displacements were used to correct displacement for anatomical differences by computing the percent of the distance or area that occurred during movement. For example,

$$\text{normalized displacement} = \frac{\text{raw displacement}}{\text{head and neck spatial measure}} \times 100\%.$$ 

However, if the intercept in the linear regression equation between the raw displacement and its corresponding anatomical distance or area measure was significantly different from 0, then this intercept was accounted for in spatial normalization. For example, $$\text{normalized displacement} = \frac{\text{raw displacement} - \text{intercept}}{\text{head and neck spatial measure}} \times 100\%.$$ 

If the linear relationship was negative and had an intercept significantly different from 0, then $$\text{normalized displacement} = \frac{\text{intercept} - \text{raw displacement}}{\text{head and neck spatial measure}} \times 100\%.$$
We then compared the coefficient of variation (standard deviation divided by the mean) of the normalized measure (%) with the raw displacement (mm). This determined if correcting for individual differences in anatomy would increase homogeneity in hyolaryngeal displacements in the swallows of healthy individuals.

Statistical analyses were conducted using IBM SPSS Statistics for Macintosh, Version 22.0 (IBM Corp, Armonk, NY).

RESULTS OF STUDY 1

Subject and swallow characteristics
Twenty-one adults (9 males) between the ages of 20 and 69 years (mean = 39) participated in Study 1. Twenty-one swallow trials were analyzed. No penetration or aspiration occurred on 15 of the swallows (PAS score 1). In 6 of the swallows, transient penetration above the level of the vocal folds was seen during swallowing, which was cleared spontaneously upon swallow completion (PAS score 2).

Measurement reliability
ICT coefficients for absolute agreement between the first and replicated sets of the 14 measures of hyoid and laryngeal displacements, vestibule closure, difference between hyoid and laryngeal elevation, and anatomy ranged from .89 to .98 (mean = .95). Absolute measurement error for each measure was as follows. LYmax: 1.5 mm; LXmax: 1.1 mm; HYmax: 1.1 mm; HXmax: 0.8 mm; HXY Area: 9.5 mm²; LYHYmaxDiff: 1.6 mm; HLarea_min: 22.0 mm²; hyoid to posterior-superior air column distance: 1.8 mm; area between hyoid and larynx: 34.8 mm²; hyoid to spine distance: 0.9 mm; hyoid to mandible distance: 1.4 mm; area representing vertical and horizontal hyoid positions: 68.6 mm²; C2-C4 distance: 0.7 mm; and, larynx to spine distance: 1.2 mm. Measures from the first dataset were used for all other analyses. Figures 4 and 5 show the distributions of these measures.
Predictors of hyoid and laryngeal displacements during swallowing

Linear regression analyses showed that LYmax during swallowing was significantly predicted by the distance between the hyoid and the posterior-superior subglottic air column \( [F(1,19) = 62.6, p < .001; \text{Table 1}] \), the area between the hyoid and larynx before swallowing \( [F(1,19) = 47.3, p < .001] \), and C2 to C4 distance \( [F(1,19) = 13.1, p = .002] \). A multiple linear regression of these anatomical measures on LYmax was significant overall \( [R^2 = .78, F(3, 17) = 20.5, p < .001] \). However, only the distance between the hyoid and the posterior-superior subglottic air column significantly predicted LYmax \( (b = 0.62, t = 2.29, p = .035, sr^2 = .07) \), while the area between the hyoid and larynx \( (b = 0.01, t = 0.65, p = .52, sr^2 = .005) \) and C2 to C4 distance \( (b = 0.20, t = 0.59, p = .56, sr^2 = .004) \) had no unique contribution in predicting LYmax.

C2 to C4 distance did not predict LXmax \( [F(1,19) = 0.7, p = .42] \), but the horizontal distance between the larynx and the spine did \( [F(1,19) = 15.6, p = .001; \text{Table 1}] \). This relationship was negative \( (r = -0.67) \), indicating that the closer the larynx was to the cervical spine, the greater the anterior laryngeal excursion during swallowing.

HYmax was significantly related to C2 to C4 distance \( [F(1,19) = 14.7, p = .001; \text{Table 1}] \), as well as the vertical distance between the hyoid and the mandible \( [F(1,19) = 11.7, p = .003] \). In the multiple linear regression analysis, both had significant unique contributions in predicting HYmax \( [\text{C2 to C4 distance: } b = 0.71, t = 3.60, p = .002, sr^2 = .26; \text{hyoid to mandible distance: } b = 0.24, t = 3.20, p = .005, sr^2 = .20] \).

HXY Area was significantly related to the area of the rectangle that represented vertical and horizontal hyoid positions at rest \( [F(1,19) = 10.3, p = .005] \). HXmax was not related to either of the anatomical measures examined (Table 1).

LYHYmaxDiff was significantly predicted by the distance between the hyoid and the posterior-superior subglottic air column \( [F(1,19) = 19.9, p < .001; \text{Table 1}] \). HLarea_min was significantly related to the area between the hyoid and larynx at rest \( [F(1,19) = 113.1, p < .001; \text{Table 1}] \).
The simple linear relationships that did not reach significance were also tested for curvilinear (quadratic) relationships and none were found to be significant.

**Relationship between laryngeal vestibule closure and hyolaryngeal displacement**

LYHYmaxDiff significantly predicted reduction in vestibule area (Fig. 6), $F(1, 19) = 45.8, p < .001, b = 12.7, SE_b = 1.9, r^2 = .71$. For every 1 mm that LYmax exceeded HYmax during swallowing, laryngeal vestibule area between the hyoid and larynx would reduce by 13 mm$^2$. LYmax also significantly predicted the extent of reduction in vestibule area, $F(1, 19) = 38.6, p < .001, b = 7.4, SE_b = 1.2, r^2 = .67$, but not LXmax [$F(1, 19) = 3.3, p = .09$], HYmax [$F(1, 19) = 6.1, p = .02$], or HXmax [$F(1, 19) = 1.3, p = .26$].

**Spatial normalization on inter-subject variability**

As LYmax, LXmax, HYmax, HXY Area, LYHYmaxDiff and HLarea_min were each predicted by one or more head and neck anatomical measures, their corresponding normalized measures were computed to correct for individual differences in anatomy. The formula to compute the normalized measure depended upon the direction of the relationship and whether the intercept was significantly different from 0 (Table 1).

Normalizing LYmax as a percentage of the distance between the hyoid and the posterior-superior air column (Fig. 7) reduced the coefficient of variation by 66 %, from 0.29 in raw LYmax (mm) to 0.10 in normalized LYmax. Normalizing LYmax by the area between the hyoid and larynx, and by C2 to C4 distance, reduced the coefficient of variation only slightly to 0.25 and 0.24 respectively. When LXmax was normalized by larynx to spine distance (Fig. 7), the coefficient of variation reduced by 60 %, from 0.53 in the raw measure to 0.21 after normalization. Normalizing HYmax as a percentage of C2 to C4 distance (Fig. 7) reduced the coefficient of variation by 68 % from 0.34 to 0.11, but normalizing by mandible to hyoid distance increased variability to 0.98. Normalizing HXY Area as a percentage of the area representing vertical and horizontal hyoid positions at rest also increased variability from 0.48 to 0.87. When
LYHYmaxDiff was normalized by the hyoid to posterior-superior air column distance at rest (Fig. 7), coefficient of variation reduced from 0.38 to 0.27. The normalized measure of HLarea_min (Fig. 7) had a reduction in coefficient of variation by 64% compared to its raw measure, from 0.44 to 0.16.

**STUDY 2**

**MATERIALS and METHODS**

**Subjects**

Healthy adults between 20 and 80 years old were recruited as volunteers separately from Study 1, and gave informed consent to participate in protocols approved by the IRBs at James Madison University and Sentara Rockingham Memorial Hospital Medical Center. Volunteers who reported the following were excluded based on screening questionnaire: swallowing difficulty, history of neurological disorder affecting swallowing function, acid reflux diagnosed by a physician, and history of head and neck cancer.

**Procedure**

The same fluoroscopic and recording equipment as those in Study 1 were used. Figure 8 shows the experiment setup. A straight metal strip was taped to the left side of the subject’s face between the tragus of the left ear and the lower border of the left eye orbit. A 6 cm long straight metal rod adapted from a hairpin was attached along the left side of the neck. The position of this rod was adjusted under fluoroscopy so that it was parallel to the cervical spine between C2 and C4. Each subject also wore a headband with a laser pointer attached to it just above the left ear, to project the laser beam onto a wall about 2.5 m opposite. The subject was instructed to keep the head in a comfortable position while seated on the fluoroscopy chair. To measure head tilt angle
relative to the cervical spine, a digital goniometer (iGaging®, St. Clemente, CA) was placed over the opening of the left external ear canal. The angle between the metal strip at the orbit and the metal rod on the neck was measured. This was the head tilt angle in neutral position (“neutral angle”). A circle 7.5 cm in diameter was attached to the wall where the laser beam projected while the subject maintained neutral head position. The subject was instructed to maintain this head position by keeping the laser beam within the boundary of this circle. A syringe containing 5 ml of thin liquid barium (Varibar®, 40% weight/volume) was then delivered orally by the examiner. The subject was reminded to keep the laser beam within the circle while holding the bolus in the mouth and throughout the swallow. The fluoroscope was then turned on and the examiner instructed the subject to “swallow now”.

Five more 5 ml thin liquid barium swallows trials were given using the same procedures as described, each in a different head tilt position from neutral, thus totaling 6 swallow trials per subject. For Trials 2 to 6, the subject was instructed to tilt the head up or down relative to the neutral angle measured in Trial 1, according to these target head tilt angles presented in randomized order: 1) 5° above; 2) 10° above; 3) 5° below, 4) 10° below, and 5) 15° below neutral angle. With each change in head tilt angle, the examiner moved the circle up or down the wall according to where the laser beam projected, and the subject used the laser light within the circle as visual feedback to minimize up and down head movement during a trial.

**Data processing**

Six swallow trials per subject were analyzed. Recordings were imported into Peak Motus 8.5 (Vicon Denver, Centennial, CO) for distance calibration, two-dimensional motion analysis and data smoothing according to the same procedures as described in Study 1.

**Measure of airway protection:** The videofluoroscopic recording of each swallow trial was rated on the Penetration Aspiration Scale (PAS) (43) to assess the integrity of airway protection.
**Kinematic measures:** Anatomical measures of hyolaryngeal positions and laryngeal vestibule size at rest before swallow onset were extracted. They were a subset of the anatomical measures in Study 1 (see Fig. 2, Points A-D, G): a) Distance between the anterior-inferior corner of the hyoid bone and the posterior-superior corner of the subglottic air column, representing vestibule length; b) Area of the space between the hyoid and larynx, representing vestibule area; c) Horizontal distance between the anterior-inferior corner of the hyoid bone and the y-axis (i.e. initial x position of the hyoid); d) Vertical distance between the anterior-inferior corner of the hyoid bone and the posterior-inferior corner of the mandibular symphysis; e) Horizontal distance between the anterior-superior corner of the subglottic air column and the y-axis (i.e. initial x position of the larynx). Additionally, 2 measures were extracted, f) Initial y position of the hyoid (i.e. the y coordinate of the anterior-inferior corner of the hyoid bone); and, g) Initial y position of the larynx (i.e. the y coordinate of the anterior-superior corner of the subglottic air column).

The following maximum displacement measures and measures of vestibule area were also derived: LYmax, LXmax, HYmax, HXmax, LYHYmaxDiff, HLarea_min, and reduction in vestibule area (Area between hyoid and larynx at rest – HLarea_min).

**Change in head tilt angle:** For each swallow trial in a different head tilt position including neutral position, head tilt angle relative to the cervical spine was derived from Peak Motus 8.5 by measuring the angle between two segments: the line connecting C2 to C4, and the line connecting the orbit to the tragus, on every frame over the time period of motion tracking. Each angle time series was smoothed using a fourth-order zero-phase Butterworth low-pass filter with a cutoff frequency of 4 Hz in Peak Motus 8.5. The mean head tilt angle was then computed for that swallow trial. For each of the 6 swallows produced by each subject, change in head tilt angle relative to the angle at neutral head position = head tilt angle – neutral angle. Thus neutral head position had a change in angle of 0°, while positive angles greater than 0° indicated higher head position relative to neutral head position, and negative angles less than 0° represented lower head
position relative to neutral. These angles varied on a continuous scale rather than in stepwise
increments or reductions of 5°, 10°, and 15° from 0°. This was because subjects did not always
produce exactly the same head tilt according to the target angle despite best efforts to keep the
head and neck as still as possible using visual feedback with the laser light. For instance, the
measured change in head tilt angle relative to neutral position from offline motion analysis might
be +4°, although the subject was guided to produce a target head tilt of +5° using the goniometer
before fluoroscopy began. Additional restraints on the subject’s head and neck to prevent any
head and neck movement during swallowing in the experimental protocol might have been
unnatural and unrepresentative of head position shifts during habitual swallowing. Therefore
angle measurements from offline motion tracking rather than those taken online during the
experiment were used in subsequent statistical analyses.

Statistical analyses
Analyses were conducted using the proc mixed command in SAS (SAS software Version 9.4 of
the SAS System for Windows).

Effects of change in head position on hyolaryngeal positions and laryngeal vestibule size at
rest. A linear mixed model was used to examine the relationship between change in head tilt
angle relative to neutral head position and each of the following 7 measures: initial x and y
positions of the hyoid, initial x and y positions of the larynx, distance between the hyoid and
posterior-superior subglottic air column at rest (representing vestibule length), area between the
hyoid and larynx at rest (representing vestibule area), and the vertical distance between the hyoid
and mandible at rest. Change in head tilt angle was entered as a fixed effect predictor. Five mixed
effects model specifications were tested in each of the 7 analyses for goodness of fit based on the
AIC (Akaike Information Criterion) statistic. The model specifications were: 1) random intercept
only; 2) random intercept and slope, with unstructured between subjects covariance; 3) no
random intercept or slope, with continuous first-order autoregressive (AR1) within subjects
covariance structure; 4) random intercept only, with continuous AR1 within subjects covariance structure; and 5) random intercept and slope, with unstructured between subjects covariance and continuous AR1 within subjects covariance structure. For the relationship between change in angle and initial x position of the hyoid, Models 2 and 5 had the lowest AICs, but the AR1 within subjects covariance estimate was 0 in Model 5. Therefore the more parsimonious Model 2 was used for null hypothesis testing and derivation of fixed and random effects estimates. For the remaining 6 relationships, Models 1 and 4 had the lowest AICs, but the AR1 within subjects covariance estimate was 0 in analyses using Model 4. Therefore, the more parsimonious Model 1 was used for null hypothesis testing and derivation of fixed and random effects estimates. A Bonferroni-corrected alpha of .007 was used to correct for multiple analyses.

**Effects of change in hyolaryngeal positions and laryngeal vestibule size at rest on hyolaryngeal displacements during swallowing.** Measures of initial hyolaryngeal positions and laryngeal vestibule length and area that were significantly predicted by change in head tilt angle were then tested for whether they predicted maximum hyolaryngeal displacements (LYmax, LXmax, HYmax, HXmax), the difference between maximum laryngeal and hyoid elevation (LYHYmaxDiff), and minimum vestibule area (HLarea_min) during swallowing. For LYmax, the possible predictors were: distance between the hyoid and posterior-superior subglottic air column, area between the hyoid and larynx and/or initial y position of the larynx. For LXmax, a possible predictor was the initial x position of the larynx. For HYmax, the possible predictors were: initial y position of the hyoid, and/or the vertical distance between the hyoid and mandible. For HXmax, a possible predictor was the initial x position of the hyoid. For LYHYmaxDiff, a possible predictor was the distance between the hyoid and posterior-superior subglottic air column. For HLarea_min, a possible predictor was the area between the hyoid and larynx at rest. Each predictor was entered univariately into a linear mixed effects model as a fixed effect. For each relationship, the 5 model specifications described above were tested for goodness of fit, and the one with the lowest AIC statistic and greatest parsimony was selected for null hypothesis
testing and derivation of fixed and random effects estimates. A Bonferroni-corrected alpha of .05 divided by the number of comparisons to predict each displacement or vestibule area measure was used to correct for multiple analyses.

**Relationship between laryngeal vestibule closure and hyolaryngeal displacement.** We investigated if reduction in the area of the vestibule from its resting area to its minimum area was predicted by the difference between the extent of laryngeal and hyoid elevation during swallowing (LYHYmaxDiff) when subjects swallowed in different head tilt positions. Five linear mixed model specifications as described above were tested for goodness of fit. The “random intercept only” model yielded the lowest AIC and was therefore used for null hypothesis testing (alpha = .05) and derivation of fixed and random effect estimates.

**RESULTS OF STUDY 2**

**Subject and swallow characteristics**

Five adults (2 males) between the ages of 53 and 66 years (mean = 62) participated in Study 2. Thirty swallow trials were analyzed, 6 from each subject. Two subjects had no penetration or aspiration on any of their swallows (PAS score 1). One subject had 5 swallows with PAS score of 1, and 1 swallow with PAS score of 2. One subject had 1 swallow with a PAS score of 1, and 5 swallows with PAS score of 2. The final subject had 5 swallows with a PAS score of 1, and 1 swallow with a score of 4 (penetration up to the level of the vocal folds, with clearance).

The anterior and posterior-superior corners of the subglottic air column could not be tracked during motion analysis in one of the swallow trials for one subject, as the shoulders obscured the view of the larynx. Therefore 29 data points were analyzed instead of 30 for measures that were computed from the x and y coordinates of the larynx.
Effects of change in head position on hyolaryngeal positions and laryngeal vestibule size at rest

Change in head tilt angle from neutral position significantly predicted the following anatomical measures before the onset of swallowing ($\alpha = .007$), (Fig. 9):

1. Hyoid $y$ position (Fixed effect of change in angle = 1.37, $SE = 0.08$, $df = 24$, $t = 17.1$, $p < .0001$; random intercept covariance estimate = 153.4, $SE = 109.3$, $Z = 1.4$, $p = .08$). Every 1° increase in head tilt angle predicted 1.4 mm upward shift of the hyoid at rest.

2. Larynx $y$ position (Fixed effect of change in angle = 1.03, $SE = 0.07$, $df = 23$, $t = 14.1$, $p < .0001$; random intercept covariance estimate = 92.2, $SE = 65.8$, $Z = 1.4$, $p = .08$). Every 1° increase in head tilt angle predicted 1 mm upward shift of the larynx at rest.

3. Distance between the hyoid and posterior-superior subglottic air column (Fixed effect of change in angle = 0.26, $SE = 0.07$, $df = 23$, $t = 3.5$, $p = .002$; random intercept covariance estimate = 41.8, $SE = 30.2$, $Z = 1.4$, $p = .08$). Every 1° increase in head tilt angle predicted 0.3 mm increase in the length of the vestibule.

4. Area between the hyoid and larynx (Fixed effect of change in angle = 3.56, $SE = 0.73$, $df = 23$, $t = 4.9$, $p < .0001$; random intercept covariance estimate = 14416, $SE = 10255$, $Z = 1.4$, $p = .08$). Every 1° increase in head tilt angle predicted 3.6 mm$^2$ increase in the area of the vestibule.

5. Vertical distance between the hyoid and mandible (Fixed effect of change in angle = 0.75, $SE = 0.12$, $df = 24$, $t = 6.1$, $p < .0001$; random intercept covariance estimate = 64.3, $SE = 47.3$, Wald $Z = 1.4$, $p = .09$). Every 1° increase in head tilt angle predicted 0.8 mm increase in the distance between the hyoid and the mandible.

There was no linear relationship between change in head tilt angle and larynx $x$ position (Fixed effect of change in angle = 0.16, $SE = 0.06$, $df = 23$, $t = 2.9$, $p = .009$; random intercept covariance estimate = 11.1, $SE = 8.2$, $Z = 1.4$, $p = .09$), or hyoid $x$ position (Fixed effect of change
in angle = 0.18, SE = 0.07, df = 4, \( t = 2.6, p = .06 \); random intercept covariance estimate range: -2.9 to 6.3, random slopes range: -0.09 to 0.14).

Effects of change in hyolaryngeal positions and laryngeal vestibule size at rest on hyolaryngeal displacements during swallowing

Three anatomical predictors of \( \text{LY}_{\text{max}} \) were tested: distance between the hyoid and posterior-superior subglottic air column, area between the hyoid and larynx, and initial \( y \) position of the larynx \( (\alpha = .017) \). For \( \text{HY}_{\text{max}} \), 2 predictors were tested: initial \( y \) position of the hyoid, and the vertical distance between the hyoid and mandible \( (\alpha = .025) \). The relationship between \( \text{LY}_{\text{HYmaxDiff}} \) and distance between the hyoid and posterior-superior subglottic air column was tested \( (\alpha = .05) \). The relationship between \( \text{HLarea}_{\text{min}} \) and the area between the hyoid and larynx at rest was tested \( (\alpha = .05) \). As \( \text{LX}_{\text{max}} \) and \( \text{HX}_{\text{max}} \) did not have any predictors, no further analyses were conducted.

The “random intercept only” linear mixed model specification yielded the lowest AIC and was used for null hypothesis testing in the following relationships. Hyoid \( y \) position before swallow onset significantly predicted \( \text{HY}_{\text{max}} \) \( (\text{Fixed effect of hyoid } y \text{ position } = -0.18, SE = 0.05, df = 24, t = -3.5, p = .002; \text{random intercept covariance estimate } = 26.2, SE = 19.2, Z = 1.4, p = .09) \), (Fig. 10A). Every 1 mm upward shift in the vertical position of the hyoid at rest predicted 0.18 mm decrease in \( \text{HY}_{\text{max}} \) during swallowing. \( \text{LY}_{\text{HYmaxDiff}} \) was significantly predicted by the distance between the hyoid and posterior-superior subglottic air column at rest \( (\text{Fixed effect of hyoid to subglottic air column distance } = 0.42, SE = 0.11, df = 23, t = 3.9, p = .0007; \text{random intercept covariance estimate } = 11.6, SE = 8.6, Z = 1.4, p = .09) \) (Fig. 10B). Every 1 mm increase in the length of the vestibule at rest predicted 0.4 mm increase in the difference between maximum laryngeal elevation and hyoid elevation. \( \text{HLarea}_{\text{min}} \) was significantly predicted by the area between the hyoid and larynx at rest \( (\text{Fixed effect of area between hyoid and larynx } = 0.51, SE = 0.08, df = 23, t = 6.0, p < .0001; \text{random intercept covariance estimate } = \)
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Every 1 mm² increase in the area of the vestibule at rest predicted 0.5 mm² increase in the minimum area of the vestibule during swallowing.

LYmax was not related to any of the 3 anatomical predictors based on corrected α of 0.017 (distance between the hyoid and posterior-superior subglottic air column: \( p = .04 \); area between the hyoid and larynx: \( p = .07 \); and initial \( y \) position of the larynx: \( p = .56 \)). The distance between the hyoid and the mandible did not predict HYmax (\( p = .83 \)).

**Relationship between laryngeal vestibule closure and hyolaryngeal displacement**

LYHYmaxDiff significantly predicted reduction in vestibule area (Fixed effect of LYHYmaxDiff = 8.5, \( SE = 1.9 \), df = 23, \( t = 4.5 \), \( p = .0002 \); random intercept covariance estimate = 1401.0, \( SE = 1068.2 \), \( Z = 1.3 \), \( p = .09 \)). For every 1 mm that LYmax exceeded HYmax during swallowing, laryngeal vestibule area would reduce by 9 mm² (Fig. 10D).

**DISCUSSION**

We investigated the anatomical factors that predicted maximum hyolaryngeal displacements and the extent of vestibule closure between the hyoid and the larynx in normal swallowing. The magnitude of laryngeal elevation was not scaled by neck length, but by how open the laryngeal vestibule was before the swallow. The extent of laryngeal elevation that exceeded hyoid elevation during swallowing, and the minimum area of the vestibule between the hyoid and the larynx were also predicted by the extent of vestibule opening at rest. Individuals adapted hyolaryngeal displacement magnitudes in response to changes in vestibule length and area at rest that were brought about by changes in head position. In addition, maximum anterior laryngeal displacement was predicted by individual differences in laryngeal position relative to the cervical spine. These relationships might have functional relevance in ensuring vestibule closure for airway protection, and forward displacement of the larynx away from the spine for UES opening. On the other hand, hyoid elevation was predicted by neck length and the vertical
position of the hyoid before the swallow. The extent of anterior hyoid displacement was not found to vary among individuals by neck length or hyoid position relative to the cervical spine.

**Predictors of laryngeal displacement**

Anatomical predictors of laryngeal displacement during swallowing have not been identified in past research; the extent of larynx-to-hyoid approximation during swallowing was unrelated to the height of an individual (25), and the distance between the mandibular symphysis and the larynx did not predict anterior laryngeal displacement (21). Here, we found that the length of the open vestibule at rest between the hyoid and the posterior larynx predicted laryngeal elevation magnitude during swallowing better than neck length (Table 1); individuals with greater laryngeal vestibule opening between the hyoid and the larynx before swallow onset elevated their larynx more during swallowing. Greater length of the vestibule before the swallow also predicted more laryngeal elevation relative to hyoid elevation. This suggests that individual differences in vestibule closure requirement not only predict the extent of laryngeal elevation by itself, but also how much laryngeal elevation is needed to overcome hyoid elevation to close the vestibule space between the hyoid and the larynx.

The extent of vestibule opening at rest may differ across people due to variation in larynx and hyoid positions. For instance, the distance between the hyoid and larynx may be larger in older individuals due to laryngeal descent with aging (24, 49). In addition, adult males may have lower larynx positions than females (8, 49). With the same goal of achieving vestibule closure to protect the airway, individuals may produce different laryngeal displacement magnitudes from one another, depending on their own anatomical requirement for vestibule closure. This may explain why large variability in laryngeal elevation magnitude has been reported across individuals (34). By correcting for individual differences in laryngeal position from the spine and the degree of vestibule opening before swallowing, variability in anterior and superior laryngeal displacement magnitudes reduced substantially by more than 60 %. This is consistent with the 56
% reduction in variability using normalized stride length in gait research (41). On the other hand, normalizing laryngeal elevation magnitude by neck length did not reduce variability substantially. Thus the extent of laryngeal displacement in normal swallowing may be scaled by how much vestibule closure is required and how much anterior laryngeal displacement away from the spine may be needed for UES opening, given underlying differences in anatomy among individuals due to factors such as age and gender.

When we systematically manipulated the degree of laryngeal vestibule opening at rest by asking each individual to swallow with different degrees of upward and downward head tilt (Fig. 9C), individuals correspondingly altered the extent to which laryngeal elevation exceeded hyoid elevation (Fig. 10B). When vestibule opening was smaller in lower head positions (Fig. 9C), laryngeal elevation did not exceed hyoid elevation by as much (Fig. 10B). This is consistent with previous studies that reported reduced hyoid to larynx distance at rest (1, 23) and reduced laryngeal elevation (23) in healthy volunteers who swallowed in chin down or chin tuck positions. However, when the airway became more exposed by increasing upward head extension (Fig. 9C), we found that the larynx elevated much more than the hyoid did, such that the difference between their displacements became larger (Fig. 10B). This increase in difference between the extent of laryngeal vs. hyoid elevation may also be contributed by reduced hyoid elevation, as the hyoid became more elevated at rest with increasing head extension upwards (Fig. 9A, 10A). These adaptations in hyolaryngeal elevation magnitudes by healthy individuals in response to changing demands for vestibule closure may be necessary, in order to consistently achieve larynx to hyoid approximation to prevent penetration. The extent of vestibule opening before the swallow therefore appears to explain both between and within subject differences in the extent to which laryngeal elevation exceeded hyoid elevation during swallowing.
**Predictors of the extent of larynx to hyoid approximation**

There was a strong positive relationship between individual differences in the area of the open vestibule between the hyoid and the larynx at rest, and the minimum area between the hyoid and the larynx during maximal vestibule closure ($r^2 = .86$, Table 1). By correcting for individual differences in vestibule size, variability among individuals reduced by 64%. Based on the normalized measure of vestibule closure during swallowing, healthy adults on average seem to approximate the larynx towards the hyoid during the peak of the swallow to about 60% of the resting area between the hyoid and the larynx (Fig. 7). When swallowing in different head positions, the area of maximal approximation between the hyoid and the larynx during swallowing also varied according to the size of the vestibule opening before the swallow (Fig. 10C). When the vestibule became more open due to a greater degree of upward head tilt (Fig. 9D), the area between the hyoid and the larynx became greater during maximal larynx to hyoid approximation (Fig. 10C). Conversely, when the head tilted downwards in a chin-down position, a correspondingly smaller vestibule area before swallowing (Fig. 9D) predicted a smaller area between the hyoid and larynx during maximal vestibule closure (Fig. 10C). From the linear mixed model analysis, every 1 mm$^2$ increase in the area of the vestibule at rest predicted 0.5 mm$^2$ increase in the minimum area of the vestibule during swallowing (i.e. 50% closure). This target of 50% closure of the vestibule relative to its resting area was similar to the 60% target among individuals (Fig. 7). Therefore, across individuals and in different swallowing contexts within an individual, there may be a consistent internal target for maximal larynx to hyoid approximation for airway protection during normal swallowing. This target may be scaled by the size of the vestibule opening before the swallow (i.e. 50 ~ 60% of the open area).

To investigate the type of movement that might be associated with larynx to hyoid approximation during swallowing, we also examined if the amount of approximation between the hyoid and the larynx during swallowing was associated with how much the larynx elevated above and beyond the extent of hyoid elevation. This relationship was significant both between and
within individuals (Fig. 6 and Fig. 10D); greater laryngeal elevation magnitude relative to hyoid elevation magnitude predicted greater reduction in the area between the hyoid and larynx during swallowing. On the other hand, anterior hyolaryngeal excursions and hyoid elevation were not associated with the extent of reduction in the area between the hyoid and larynx. This suggests that adaptation by individuals in the extent of laryngeal elevation relative to hyoid elevation may be important to ensure adequate larynx to hyoid approximation for vestibule closure.

**Predictors of hyoid displacement**

Individual difference in hyoid elevation magnitude in normal swallowing was significantly explained by both neck length and hyoid height. However, neither neck length nor hyoid position predicted the extent of anterior hyoid excursion. These findings agree with those previously reported (20, 35). Normalizing superior hyoid displacement by neck length reduced inter-subject variability substantially, but normalizing by hyoid height increased variability. Therefore the size of the neck in which swallowing occurs may be a contributing factor to variability in hyoid elevation. On the other hand, when individuals swallowed in different head positions, the extent of hyoid elevation during swallowing was predicted by vertical hyoid position; less elevation occurred when the hyoid was higher in the neck before swallowing (Fig. 10A). A possible explanation is that suprathyroid muscles attached to the mandible and floor of the mouth may already be in a contracted state to maintain an elevated hyoid position at rest. As the extent of shortening of the suprathyroid muscles may correlate with the magnitude of hyoid elevation during swallowing, reduced shortening of these muscles that are already contracted may contribute to reduced hyoid elevation (38). Another reason for reduced hyoid elevation when its baseline position is higher may be to ensure that the larynx can still approximate the hyoid sufficiently to close the vestibule. In this case, individuals may also adapt hyoid elevation magnitude depending on the extent of approximation with the larynx needed for vestibule closure.
Neither neck length nor hyoid position from the cervical spine correlated with anterior hyoid displacement. This may be because anterior hyoid excursion is less variable than superior hyoid excursion in healthy individuals (13, 34). Ishida et al. (13) proposed that the extent of anterior hyoid excursion required for UES opening may be consistent across individuals, thus contributing to the small variance in this measure. Others have proposed that individuals with a shorter distance between the chin and the spine, or a lower chin position relative to the cranium would exhibit smaller anterior hyoid displacements during swallowing (20, 32). However, we did not find significant changes in anterior hyoid excursion during swallowing when the chin position changed with different head positions.

**Goal-directed movement scaling in swallowing**

Laryngeal vestibule closure and UES opening are important for safe swallowing. Thyrohyoid muscle contraction is thought to contribute to superior and anterior laryngeal movement, resulting in the approximation of the larynx to the hyoid for laryngeal vestibule closure (2, 7, 27, 39, 47). UES opening may be contributed by a series of coordinated events—reflexive UES relaxation controlled by the brainstem (54), laryngeal elevation to the hyoid and rapid anterior hyoid excursion that pulls the larynx forward (4, 14, 51, 52). Overall, our results suggest that the length or area of the laryngeal vestibule that requires closure during swallowing, the position of the larynx relative to the spine, and hyoid height may be more relevant than neck length to the functions of vestibule closure and UES opening for safe swallowing. Therefore, these measures of vestibule size and hyolaryngeal positions at rest predicted the extent of hyolaryngeal elevation, anterior laryngeal displacement and the amount of closure between the hyoid and the larynx during swallowing. Exceptions were in the positive relationship between neck length and hyoid elevation magnitude between individuals, and the lack of systematic variation in anterior hyoid displacement by anatomical difference or change in head and hyoid positions. Laryngeal movement magnitudes for swallowing may follow the principle of goal-
oriented movement scaling reported in the speech motor control literature (9, 42). In speech, displacements of the articulators are correlated with the distance required to approximate the articulatory target such as bilabial closure, rather than the overall size of the articulatory system (42). In swallowing, the movement target for upward and forward laryngeal motion may be to approximate the hyoid to achieve laryngeal vestibule closure, and to displace away from the cervical spine for UES opening. This target may differ across people due to anatomy based on the results of Study 1, or alter within individuals when vestibule closure requirement changes under different swallowing conditions, based on the results of Study 2. The individual may then adapt by adjusting hyolaryngeal elevation magnitudes so that the extent of laryngeal elevation always exceeds hyoid elevation to a degree that achieves vestibule closure. This effect was also demonstrated by Humbert et al. (11), who found that healthy individuals adapted to lowering of the larynx induced by electrical stimulation, by increasing the extent of laryngeal elevation against resistance to exceed the concurrent increase in hyoid elevation against resistance.

Individuals swallow from birth and implicitly adapt their swallowing system to anatomic changes with development (26), and when eating and drinking foods and liquids of different amounts and textures (13, 18). Sensory feedback may be crucial in the implicit modulation of the swallow motor response (12), and this may contribute to adaptation in hyolaryngeal displacement magnitudes when changes in swallowing conditions are anticipated (11). The laryngeal vestibule that is bordered by the arytenoids and the laryngeal surface of the epiglottis contains high densities of slowly and rapidly adapting afferent fibers of the internal branch of the superior laryngeal nerve (iSLN) (5, 36, 53). Discharges from the iSLN also appear to increase during laryngeal elevation and thyrohyoid muscle contraction (44). This anatomical framework may contribute to the gradual adaptation of the swallowing motor pattern to laryngeal posture changes during development and aging, and may facilitate rapid response to material entering the vestibule through coughing. Deprivation of laryngeal sensory feedback may be detrimental to swallow safety. Jafari et al. (15) found that bilateral sensory block of the iSLN in healthy
volunteers increased the frequency of silent penetration and resulted in aspiration in 25% of the swallows. The episodes of penetration occurred not just in the beginning but throughout the duration of anaesthesia, without alterations in the durations of laryngeal closure and apnea during swallowing (15). Evidently, slow and rapid adaptations of the swallowing response for airway protection might have been impeded due to diminished laryngeal sensory feedback to the central nervous system. This suggests the importance of an intact afferent system in facilitating hyolaryngeal movement scaling for safe swallowing.

**Study limitations**

A limitation in this study is that the intercept and slope of the linear equation representing the relationship between the anatomical measure and the displacement/area measure were based on the line of best fit for this sample of healthy individuals. These may change with other swallowing and subject samples and therefore alter the mathematical computation of the normalized displacement measure.

**Conclusion**

Goal-directed movement scaling, which is found in other areas of skilled motor control such as speech, was predominant in explaining the extent of hyolaryngeal displacements in normal swallowing. Larynx to hyoid approximation for vestibule closure and forward laryngeal displacement away from the cervical spine for UES opening are two important movement goals for swallowing. These movement goals likely explain how individual differences in vestibule size and hyolaryngeal positions at rest predicted the extents of hyolaryngeal displacements and vestibule closure during swallowing. Under swallowing conditions that altered laryngeal vestibule opening before swallow onset, individuals also adapted the extent of hyoid and laryngeal elevation so that laryngeal elevation could override hyoid elevation to meet the requirement amount of vestibule closure. This adaptation may be possible as years of continual swallowing experience may allow individuals to implicitly develop an internal model of
swallowing. This internalized pattern may include sensory feedback on the extent of hyolaryngeal movement required for vestibule closure and UES opening.

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## TABLES

Table 1. Results of simple linear regressions

<table>
<thead>
<tr>
<th>Measure Y</th>
<th>Predictor X</th>
<th>r (r^2)</th>
<th>Equation Y = bX + c</th>
<th>SE_b</th>
<th>p</th>
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<tr>
<td>LYmax</td>
<td>Hyoid to posterior-superior air column distance</td>
<td>.88 (.77)</td>
<td>Y = 0.86X - 9.5 †</td>
<td>0.11</td>
<td>&lt;.001 *</td>
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<td></td>
<td>Area between hyoid and larynx</td>
<td>.85 (.71)</td>
<td>Y = 0.04X + 10.0 †</td>
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<td>&lt;.001 *</td>
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<td></td>
<td>C2-C4 distance</td>
<td>.64 (.41)</td>
<td>Y = 1.3X - 22.9</td>
<td>0.37</td>
<td>.002 *</td>
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<td>LXmax</td>
<td>Larynx to spine distance (horizontal)</td>
<td>-.67 (.45)</td>
<td>Y = -0.46X + 21.7 †</td>
<td>0.12</td>
<td>.001 **</td>
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<tr>
<td></td>
<td>C2-C4 distance</td>
<td>.19 (.04)</td>
<td>Y = 0.18X - 0.1</td>
<td>0.22</td>
<td>.42</td>
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<tr>
<td>HYmax</td>
<td>Mandible to hyoid distance (vertical)</td>
<td>.62 (.38)</td>
<td>Y = 0.31X + 8.7 †</td>
<td>0.09</td>
<td>.003 **</td>
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<td></td>
<td>C2-C4 distance</td>
<td>.66 (.44)</td>
<td>Y = 0.88X - 18.3 †</td>
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<td>.001 **</td>
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<td>HXmax</td>
<td>Hyoid to spine distance (horizontal)</td>
<td>-.28 (.08)</td>
<td>Y = -0.18X + 19.8 †</td>
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<td>.33</td>
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<td>HXY Area</td>
<td>Area representing vertical and horizontal hyoid positions</td>
<td>.59 (.35)</td>
<td>Y = 0.06X + 46.0 †</td>
<td>0.02</td>
<td>.005 ***</td>
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<td>LYHYmaxDiff</td>
<td>Hyoid to posterior-superior air column distance</td>
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<td>Y = 0.42X - 5.5</td>
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<td>&lt;.001 ***</td>
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<tr>
<td>HLarea_min</td>
<td>Area between hyoid and larynx</td>
<td>.93 (.86)</td>
<td>Y = 0.62X - 4.0</td>
<td>0.06</td>
<td>&lt;.001 ***</td>
</tr>
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†   Intercept significantly different from 0, p < .05
*  Significant using corrected α = .017
** Significant using corrected α = .025
*** Significant using corrected $\alpha = .05$
FIGURE LEGENDS

Figure 1.
Left lateral view of a fluoroscopic video frame. The $y$-axis intersects the anterior-inferior corners of the 2nd (C2) and 4th (C4) cervical vertebrae. The $x$-axis is at 90° to $y$ and intersects the anterior-inferior corner of C4. Hyoid (anterior-inferior corner of the hyoid bone) and larynx (anterior-superior corner of the subglottic air column) positions are tracked in the $x$ and $y$ dimensions during swallowing. The hyoid bone and the superior aspect of the subglottic air column are outlined.

Figure 2.
Left lateral view of a fluoroscopic video frame showing structures tracked in motion analysis. The numbers indicate measurement points: 1) anterior-inferior corner of C2; 2) anterior-inferior corner of C4; 3) anterior-inferior corner of the hyoid bone; 4) anterior-superior corner of the subglottic air column; 5) posterior-superior corner of the subglottic air column; 6) posterior-inferior corner of the mandibular symphysis.

The letters indicate anatomical distance and area measures obtained from the first frame that was tracked in each video: A) Distance between anterior-inferior corner of the hyoid bone (3) and posterior-superior corner of the subglottic air column (5); B) Area of the triangle bound by Points 3 to 5 [the anterior-inferior corner of the hyoid bone (3), anterior-superior corner of the subglottic air column (4) and posterior-superior corner of the subglottic air column (5)]; C) Horizontal distance between the anterior-inferior corner of the hyoid bone (3) and the $y$ axis; D) Vertical distance between the anterior-inferior corner of the hyoid bone (3) and the horizontal line connecting the posterior-inferior corner of the mandibular symphysis (6) perpendicularly to the $y$ axis; E) Area of the rectangle bound by length C and height D; F) Distance between the anterior-
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Anterior hyoid displacement across time in a subject, where displacement at the first data point = 0. Maximum anterior hyoid displacement (HXmax) is the difference between the maximum y and initial positions. Line (a) is the time when the bolus head reached the angle of the mandible. Line (b) represents the time when the tail of the bolus passed the level of the 6th cervical vertebra (C6). Motion tracking began 1 s before time (a) and ended 1 s after time (b).

Figure 4.
Boxplots showing the distributions of raw measures of displacement (LYmax, LXmax, HYmax, HXmax, LYHYmaxDiff) and area (HLarea_min, HXY Area) during swallowing across 21 healthy volunteers.

Figure 5.
Boxplots showing the distributions of anatomical measures at rest across 21 healthy volunteers.

Figure 6.
Relationship between the extent of laryngeal elevation that exceeded hyoid elevation (LYHYmaxDiff), and change in vestibule area from resting area to minimum area during swallowing across 21 healthy volunteers.

Figure 7.
Boxplots showing the distributions of normalized measures of displacement across 21 healthy volunteers. From left: LYmax normalized by distance between hyoid and posterior-superior
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Figure 8.
Schematic drawing (not to scale) of the experimental setup for swallow trials in different head tilt positions.

Figure 9.
Relationships between change in head tilt angle relative to neutral position, and A) Initial y position of the hyoid at rest, B) Initial y position of the larynx at rest, C) hyoid to posterior-superior subglottic air column distance at rest, D) area between hyoid and larynx at rest, and E) Vertical distance between hyoid and the mandible at rest. Measures were obtained from 5 subjects (represented by different symbols) who each swallowed in 6 different head tilt positions. The trendline for each subject is shown on each scatterplot.

Figure 10.
Relationships between anatomical and displacement/vestibule area measures across 5 subjects (represented by different symbols) who swallowed in 6 different head tilt positions. The overall group trendline is shown on each scatterplot, but intercepts were allowed to vary across subjects as the random effect of intercept was modeled in each analysis. A: Relationship between maximum hyoid elevation (HYmax) and initial y position of the hyoid at rest. B: Relationship between the extent of laryngeal elevation exceeding hyoid elevation (LYHYmaxDiff) and hyoid to posterior-superior subglottic air column distance at rest. C: Relationship between minimum vestibule area during swallowing (HLarea_min) and vestibule
area at rest (area between hyoid and larynx). D: Relationship between the extent of laryngeal elevation exceeding hyoid elevation (LYHYmaxDiff) and the reduction in vestibule area between resting and minimum areas during swallowing.
FIGURES

Figure 1.
Figure 2
Figure 3.
Figure 4

Raw displacement (mm)

Raw area (mm²)

LYmax  LXmax  HYmax  HXmax  LYHYmaxDiff

HLarea_min  HXY Area
Figure 6.

$R^2$ Linear = 0.707
Figure 7.

Normalized displacement (%) 

Normalized area (%) 

LYmax  LXmax  HYmax  LYHYmaxDiff
Figure 8

Laser beam shines onto this circle

Laser pointer on headband

Back of chair

Wall about 2.5 m away from subject

Metal strip between orbit and tragus

Metal rod parallel to C2-C4

Calibration ball
Figure 9.
Figure 10.

A

B

C

D

Reduction in vestibule area (mm²)

Hyoid y position at rest, mm (relative to origin at C4)

Hyoid to posterior subglottic air column distance (mm)

Htarea_min_during_swallowing (mm²)

vestibule area at rest (mm²)

Reduction in vestibule area (mm²)

LHYmaxDiff (mm)
TITLE
Reduced laryngeal elevation characterizes patient swallows using anatomically scaled hyolaryngeal displacement measures

RUNNING HEAD
Reduced laryngeal elevation characterizes patient swallows
ABSTRACT

Laryngeal vestibule closure and upper esophageal sphincter opening (UES) are important for safe swallowing. Despite impairment in vestibule closure and UES opening, patients with dysphagia may exhibit reduced, increased or similar hyolaryngeal displacements as healthy individuals. We investigated if hyolaryngeal maximal displacements that corrected for individual differences in anatomy would show greater differences between the swallows of patients and healthy individuals than uncorrected measures. We also examined if the relationship between hyoid and laryngeal elevation would differentiate between patients and controls swallowing. Single swallows recorded during videofluoroscopy from 21 healthy volunteers and 21 patients were analyzed using 2D motion analysis of hyoid and laryngeal movements. Spatially normalized measures of hyoid and laryngeal elevation magnitudes showed greater differences between normal and abnormal swallowing than raw measures. The extent of laryngeal elevation and anterior hyoid displacement were more important than that of hyoid elevation in differentiating between normal and abnormal swallowing. The difference between maximum laryngeal and hyoid elevation magnitudes was negative in patients’ swallows, indicating they had insufficient laryngeal elevation relative to hyoid elevation to achieve vestibule closure during swallowing. Neither raw nor normalized displacement measures differed between patient swallows with and without UES opening. In conclusion, when hyoid elevation is greater than laryngeal elevation, it can be detrimental to airway protection for swallowing in dysphagia.

KEYWORDS

Deglutition, dysphagia, hyoid movement, larynx movement, swallow outcome measure
INTRODUCTION

In swallowing, safe transport of food and liquid through the pharynx into the esophagus depends on laryngeal vestibule closure and the opening of the upper esophageal sphincter (UES). The hyoid bone and the larynx may have overlapping but distinct roles in these two mechanisms. Superior and anterior laryngeal displacements are needed to shorten the distance between the hyoid and the larynx for vestibule closure and to allow the epiglottis to fold over the ascending larynx (4, 14). On the other hand, both hyoid and laryngeal movements may contribute to UES opening. Superior hyoid and laryngeal excursion are thought to occur first (3, 28), followed by rapid anterior hyoid movement that pulls on the larynx to exert forward traction on the anterior aspect of the UES (3, 6, 28, 29). Superior laryngeal elevation may also aid UES opening by stretching the UES from the posterior sphincter wall (6).

Given these crucial functions of hyolaryngeal movements in normal swallowing, one would expect patients with dysphagia, who often present with reduced vestibule closure and limited UES opening, to exhibit reduced hyolaryngeal displacements in swallowing. Both reduced anterior and superior hyolaryngeal displacement may increase the risk of penetration and aspiration (1, 23). However, patients had increased (10, 12), decreased (27) or similar (1, 20, 24) maximal hyolaryngeal displacement magnitudes during swallowing when compared with healthy individuals. Furthermore, hyoid displacement has not consistently improved with dysphagia intervention (25).

Normalizing hyoid elevation by individual differences in neck length may differentiate patients with different severities of dysphagia (21). However, hyoid and laryngeal displacements normalized by neck length failed to distinguish between patients with and without bolus penetration into the airway (23). It is not known if measures of hyolaryngeal displacements normalized by other differences in anatomy would better differentiate between normal and abnormal swallowing. Without measures of hyolaryngeal impairment that characterize
swallowing abnormality, the detection of changes in hyolaryngeal kinematics due to intervention, spontaneous recovery or disease progression is limited (13).

In this study, we examined whether improved differentiation between normal and disordered swallows would occur with measures of hyolaryngeal displacement that correct for differences in anatomy, and the relationship between hyoid and laryngeal elevation for vestibule closure. In addition, we examined whether such measures could also distinguish between different severities of dysphagia by comparing swallows with and without bolus passage through the UES. Finally, we examined the relationships between hyolaryngeal displacement and penetration/aspiration severity. In a previous study in healthy adults, we found that anatomical measures such as neck length, and the distance between the hyoid and the posterior larynx forming the vestibule significantly predicted the magnitudes of hyoid and laryngeal elevation during swallowing (Wong et al., in preparation). In the same study, we found that the distance between the larynx and the cervical spine predicted laryngeal anterior displacement.

Here, we hypothesized that when hyoid and laryngeal movements are normalized for differences in anatomical requirements for safe swallowing, they would more accurately discriminate between normal and disordered swallowing than raw displacement measures. We expected that normalized measures would differentiate between swallows with and without UES opening in patients and that spatially normalized displacements would correlate with penetration/aspiration severity. Finally, we used discriminant function analyses to determine the accuracy of measures for differentiating between swallows from healthy volunteers and patients with dysphagia.
MATERIALS and METHODS

Subjects

Adults between 20 to 80 years old were recruited as healthy volunteers and gave informed consent to participate in a protocol approved by the Institutional Review Boards (IRBs) at James Madison University and Sentara Rockingham Memorial Hospital Medical Center. Healthy volunteers were excluded if they reported: swallowing difficulty, history of a neurological disorder affecting swallowing function, acid reflux diagnosed by a physician, or history of head and neck cancer. De-identified archived video recordings gathered from healthy subjects under IRB approved protocols from the National Institute of Neurological Disorders and Stroke were also included.

Patients seen in the Voice and Swallow Services at Sentara Rockingham Memorial Hospital Medical Center and research participants in other swallowing studies at James Madison University were also recruited and gave their informed consent to participate. They were included if they were above the age of 12, and had dysphagia either following treatment for head and neck cancer or neurological disorders such as stroke. There were no restrictions on the time since onset of their medical diagnosis or dysphagia. We also used de-identified archived video recordings of patients with chronic dysphagia either due to stroke or following treatment for head and neck cancer. These recordings were obtained under IRB approved protocols at the National Institute of Neurological Disorders and Stroke and met the above inclusion criteria.

Procedure

A radio-opaque ball with a 19 mm diameter was taped to the side of the subject’s neck posterior to the spine for converting pixels into millimeters. A digital Siemens fluoroscope (Model AXIOM Luminos TF) was set up for a lateral view from anterior neck extending inferiorly from the trachea and below the upper esophageal sphincter, to posterior spine from C1 to C6 and extending superiorly to the floor of the nasal cavity (Fig. 1). A syringe containing 5 ml
of thin liquid barium (Varibar®, 40% weight/volume) was delivered orally by the examiner, except for 7 patients who received the 5 ml liquid barium via spoon. The fluoroscopy was turned on and the examiner instructed the participant to “swallow now”. Magnification was unchanged throughout the swallow. Each fluoroscopic swallow trial was captured at 30 frames/s and saved in .avi format using a D-scope® System (D-scope® Systems, Brooklyn, NY).

To determine if swallow trials from patients could be pooled together regardless of the bolus delivery method, 10 additional patients who were referred to the Voice and Swallowing Services for a modified barium swallow assessment were recruited into the study and gave their informed consent to participate. No age or medical diagnosis limits were applied for inclusion. These patients underwent videofluoroscopy with the same equipment and instructions as the patients and healthy volunteers, except they each received two 5 ml thin liquid barium boluses, one using a syringe and the other using a spoon in randomized order across the 10 patients.

Data processing

Videofluoroscopic recordings were imported into Peak Motus 8.5 (Vicon Denver, Centennial, CO) for distance calibration and two-dimensional motion analysis. One swallow trial per subject was analyzed, except for the 10 patients who participated in one syringe-delivered and one spoon-delivered liquid swallow trial.

Conversion into millimeters: To convert pixels into millimeters, the diameter of the calibration ball was measured on one video frame in the recording. For consistency in frame selection across videos, we used the frame when the head of the bolus reached the angle of the mandible. If motion blur occurred on this frame due to subject movement, then another video frame with adequate clarity and contrast of the calibration ball within the swallow sequence was selected. As fluoroscopic magnification was unchanged during the swallow trial, the same scaling factor was automatically applied to the other frames in the same recording.
Spatial analysis: The anterior-inferior corner of C4 served as the origin for the $x$ and $y$-axes in the horizontal and vertical dimensions respectively (Fig. 1). The $y$-axis connected the origin to the anterior-inferior corner of C2, while the $x$-axis was perpendicular to the $y$-axis at the origin (Fig. 1). A spatial model of the measurement points for motion analysis was set up in Peak Motus 8.5 to manually track the position of each point frame by frame using a cursor. These points were: 1) Anterior-inferior corner of C2; 2) Anterior-inferior corner of C4; 3) Anterior-inferior corner of the hyoid bone; 4) Anterior-superior corner of the subglottic air column to track the larynx; and, 5) Posterior-superior corner of the subglottic air column. Distances between measurement points were also derived for each frame (Fig. 2).

Time periods measured: Measurement for each swallow started on the frame that was 1s before the time when head of the bolus reached the angle of the mandible. However, if the hyoid and larynx had already begun movement at this time point, then motion analysis was begun further back in time closer to the start of the fluoroscopic recording to capture the resting positions of the hyoid and larynx while the bolus was held in the oral cavity. For healthy volunteers, motion tracking continued until 1s after the tail of the bolus passed the anterior-inferior corner of C6. However, many of the patients swallowed multiple times per bolus and some had insufficient UES opening for the bolus to flow past the level of C6. Therefore, motion tracking of the swallow trials of all patients continued until any of the following events occurred: onset of a second swallow, cessation of fluoroscopy, or when excessive motion blur accompanied patient movement (e.g. during coughing).

Filtering the kinematic time series data: A fourth-order, zero time lag Butterworth low-pass filter with a cutoff frequency of 4 Hz was applied within Peak Motus 8.5 to smooth the kinematic data for $x$ and $y$ over time. As the filter made recursive passes in forward and backward directions, time lag was not expected in the filtered data. The smoothed position and segmental distance time series data were exported into Matlab R2013a (The Mathworks, Inc., Natick, MA).
Anatomical measures made at rest before swallowing onset (Fig. 2): These measures were derived from the first data point at rest in the smoothed positions over time (i.e. at least 1s before the time when head of the bolus reached the angle of the mandible).

A) Distance between the anterior-inferior corner of the hyoid bone and the posterior-superior corner of the subglottic air column (HLdist) (mm). This represented the opening of the laryngeal vestibule at rest. The posterior rather than anterior corner of the subglottic air column was used, as this point represented the position of the larynx and the cricopharyngeus muscle or UES before swallowing.

B) Horizontal distance between the anterior-superior corner of the subglottic air column and the y-axis (Larynx to spine distance) (mm) (i.e. the x coordinate of position of the anterior-superior corner of the subglottic air column). This represented larynx position in the anterior-posterior plane relative to the spine.

C) Distance between C2 and C4 (C2-C4 distance) (mm) represented neck length.

D) Area of the space between the hyoid and larynx (HLarea) (mm²). This was calculated by applying Heron’s formula (26) to the triangle bound by the anterior-inferior corner of the hyoid bone, the anterior-superior corner of the subglottic air column and the posterior-superior corner of the subglottic air column. This represented the area of the laryngeal vestibule between the hyoid and the larynx at rest.

Raw displacement measures from kinematic data (mm): The initial positions of the hyoid and larynx were linearly transposed so that all initial positions (i.e. the first data point) had a displacement of 0 mm. The following displacement measures were computed.

Maximum superior laryngeal displacement (LYmax) = difference between the maximum and initial positions in the smoothed y over time of the anterior-superior subglottic air column (Fig. 3).

Maximum anterior laryngeal displacement (LXmax) = difference between the maximum and initial positions in the smoothed x over time of the anterior-superior subglottic air column.
Maximum superior hyoid displacement (HYmax) = difference between the maximum and initial positions in the smoothed y over time of the anterior-inferior corner of the hyoid bone.

Maximum anterior hyoid displacement (HXmax) = difference between the maximum and initial positions in the smoothed x over time of the anterior-inferior corner of the hyoid bone.

For patients, the above measures were obtained from the first swallow attempt, regardless of whether or not the bolus passed through the UES in that attempt. Thus comparisons were made with first swallows of healthy volunteers who cleared the bolus through the UES. The end of the first swallow attempt in patients was defined as the time when the larynx reached its lowest y position after peak elevation (Fig. 4). This time point was chosen as the lowest larynx position usually occurred when the upper airway resumes its role in respiration. Using the conventional definition of swallow offset as the passage of the bolus through the UES (11) was not possible in patients as many did not have UES opening.

Spatially normalized displacement measures: In a separate study (Wong et al., in preparation), we found significant linear relationships between HLDist and LYmax, between larynx to spine distance and LXmax, and between C2-C4 distance and HYmax in 21 healthy volunteers. The linear equations of these relationships were: predicted LYmax = 0.86*HLDist – 9.549; predicted LXmax = -0.46*larynx to spine distance + 21.693; and, predicted HYmax = 0.88*C2-C4 distance - 18.316. These equations showed the expected maximum displacement magnitude in mm for a healthy adult after correcting for individual differences in anatomy measured in mm. For instance, a healthy individual with HLDist = 40 mm (length of the vestibule at rest) will have an expected LYmax of 25 mm (LYmax=(0.86*40)-9.549=25) We did not find anatomical measures that significantly predicted HXmax (Wong et al., in preparation); therefore no linear equation was available to predict HXmax.

The above relationships were used to convert raw displacements into the percent of an anatomical measure for each individual, by computing what percent of the anatomical measure occurred during movement. For example,
normalized displacement = \frac{raw displacement - intercept}{anatomical measure} \times 100 \%, if the relationship between
the raw displacement and anatomical measure was positive (e.g. between LYmax and HLdist,
and between HYmax and C2-C4 distance). On the other hand, normalized displacement =
\frac{intercept - raw displacement}{anatomical measure} \times 100 \%, if the linear relationship was negative (e.g. between LXmax
and larynx to spine distance). We used the following 3 formulas to normalize displacements by
individual differences in anatomy for the swallows of healthy volunteers and patients in this
study, so that comparisons could be made between the groups. There was no normalized measure
of HXmax as it was found to have no significant anatomical predictor (Wong et al., in
preparation).

Normalized maximum superior laryngeal displacement (normLYmax):

\quad \text{normLYmax} = \frac{LYmax + 9.549}{HLdist} \times 100 \%

Normalized maximum anterior laryngeal displacement (normLXmax):

\quad \text{normLXmax} = \frac{21.693 - LXmax}{larynx to spine distance} \times 100 \%

Normalized maximum superior hyoid displacement (normHYmax):

\quad \text{normHYmax} = \frac{HYmax + 18.316}{C2 to C4 distance} \times 100 \%

Measures of laryngeal vestibule closure and relationship between hyoid and laryngeal
elevation during swallowing:

Minimum area between hyoid and larynx (HLarea_min) (mm²). This represented the minimum
area of the laryngeal vestibule between the hyoid and larynx achieved during a swallow. This was
derived by applying Heron’s formula (26) to the time series of the segmental distances between
these 3 points: 1) anterior-inferior corner of the hyoid bone, 2) anterior-superior corner of the
subglottic air column, and, 3) posterior-superior corner of the subglottic air column (Fig. 2), and
then identifying the minimum value. In a separate study (Wong et al., in preparation), we found that HLarea significantly predicted HLarea_min (predicted HLarea_min = 0.62*HLarea – 4.0).

Normalized minimum area between hyoid and larynx (normHLarea_min) (%). This represented the minimum area of the laryngeal vestibule between the hyoid and larynx during swallowing, after correcting for individual differences in HLarea (mm²) at rest: normHLarea_min =

\[
\frac{HLarea_{min}}{HLarea} \times 100\%
\]

Normalized difference between laryngeal elevation and hyoid elevation (normLYHYmaxDiff) (%). This was the difference between normLYmax (%) and normHYmax (%).

**Measures obtained from spoon vs. syringe delivered swallow trials:** Recordings from the 10 patients who participated in the comparison between spoon and syringe-delivered trials were processed in the same way as the other patients. Only the measures of HXmax, HYmax, LXmax and LYmax, and the initial x and y positions of the hyoid and larynx at rest were extracted for spoon and syringe swallow comparisons.

**Statistical analyses**

**Comparison of spoon vs. syringe delivery method:** Paired t-tests (2-tailed) were conducted to examine the differences in initial hyoid and laryngeal x and y positions after administration by spoon or by syringe, and displacement during swallows (HXmax, HYmax, LXmax and LYmax) between the two delivery methods. Statistical significance was set at the uncorrected level of \( \alpha = .05 \) to reduce Type 2 error.

**Measurement reliability:** The same investigator replicated measures from healthy volunteers and patients for intra-rater reliability. The intra-class correlation coefficient (ICC) was computed based on a two-way random effects model (assuming the effects of subject and swallow trial were random) for each of the following 13 measures: HLdist, larynx to spine distance, C2-C4 distance, LYmax, LXmax, HYmax, HXmax, normLYmax, normLXmax, normHYmax, HLarea_min, normHLarea_min, and normLYHYmaxDiff. The absolute measurement error in mm (relative
difference between the first and replicated measure) was also computed for each measure. The first data set was used in subsequent analyses.

**Comparisons between swallows of healthy volunteers and patients with dysphagia:** Each of the swallows produced by healthy volunteers, and by patients, was rated on the Penetration-Aspiration Scale (PAS) (22) to assess the integrity of airway protection. As PAS scores were skewed in healthy volunteers and therefore not normally distributed, the non-parametric Mann-Whitney test (2-tailed) was used to compare swallows of healthy volunteers and patients with dysphagia ($\alpha = .05$).

To determine if the healthy volunteers and patients differed in anatomical distances, and if their swallows differed on measures of hyolaryngeal displacement and vestibule closure, one-way analyses of variance (ANOVAs) were conducted on 13 measures, using a Bonferroni-corrected $\alpha$ of 0.004: on anatomical measures at rest (HLdist, larynx to spine distance, and C2-C4 distance); raw displacements (LYmax, LXmax, HYmax, and HXmax); normalized displacements (normLYmax, normLXmax, and normHYmax); and measures of laryngeal vestibule closure (HLarea_min, normHLarea_min, and normLYHYmaxDiff). To determine if normalized displacement measures (normLYmax, normLXmax, normHYmax) could differentiate between swallows produced by healthy volunteers and patients with dysphagia, a discriminant function analysis was conducted with $\alpha$ set at 0.05.

To determine the relationship between penetration/aspiration and normalized measures of hyolaryngeal displacement and vestibule closure, PAS scores were correlated with normLYmax, normLXmax, normHYmax, normHLarea_min, and normLYHYmaxDiff. Statistical significance was set at $\alpha = .01$ to correct for multiple analyses.

**Comparisons between patient swallows:** Swallows produced by patients that were classified as without UES opening did not have any bolus pass through the UES. Those that had some of the bolus, even if it was a small amount, passing through the UES were classified as swallows with
UES opening. A Mann-Whitney test (2-tailed) determined if PAS scores differed between the swallows with UES opening from those without ($\alpha = .05$).

To determine if patient swallows with UES opening differed from those without UES opening, ANOVAs were conducted on each of the 13 measures, using a Bonferroni-corrected $\alpha$ of 0.004: anatomical measures at rest (HLdist, larynx to spine distance, and C2-C4 distance); raw displacements (LYmax, LXmax, HYmax, and HXmax); normalized displacements (normLYmax, normLXmax, and normHYmax); and measures of laryngeal vestibule closure (HLarea_min, normHLarea_min, and normLYHYmaxDiff).

Statistical analyses were conducted using IBM SPSS Statistics for Macintosh, Version 22.0 (IBM Corp, Armonk, NY).

RESULTS

Subject characteristics

Twenty-one healthy adults (9 males) who ranged from 20 to 69 years old (mean = 39) participated as healthy volunteers. They were also participants in another study reported in a separate paper (Wong et al., in preparation). The swallows of the healthy volunteers did not evidence dysphagia based on Penetration-Aspiration Scale scores, and the investigator’s clinical judgment on viewing the videofluoroscopic recordings.

Twenty-one patients (16 males) between 20 and 82 years old (mean = 64) were included in this study. They had medical diagnoses of neurological disorders (9 patients) or head and neck cancer (12 patients). Patients’ swallows evidenced dysphagia based on Penetration-Aspiration Scale scores, and the clinical judgment of the investigator on viewing the videofluoroscopic recordings.

Ten patients (4 males, mean age = 74, range from 47 to 96 years old) consented to participate in the comparison between spoon and syringe bolus delivery. Their swallow trials
were not included for analysis with those of the other 21 patients, as either the participant did not have neurological disorder or head and neck cancer (6 patients), or their swallows were normal on the 5 ml liquid swallows on videofluoroscopy (8 patients).

**Comparison of swallows with spoon vs. syringe delivery**

Paired t-tests showed no significant differences using $\alpha = .05$, between spoon and syringe bolus delivery on $HX_{max}$, $HY_{max}$, $LX_{max}$ and $LY_{max}$, or in the initial $x$ and $y$ positions of the hyoid and the larynx at rest after presentation of the bolus by spoon or syringe. The $p$ values ranged from .17 to .79. Therefore, swallow trials of the 21 patients were pooled together for analysis regardless of whether the 5 ml liquid bolus was delivered by spoon or by syringe.

**Measurement reliability**

When measures from 42 swallow trials (from 21 healthy volunteers and 21 patients) were replicated, the ICC coefficients for intra-rater reliability of the 13 measures were between .84 and .98. Absolute measurement error between the two sets of measures were: 1.6 mm (HLdist), 1.2 mm (larynx to spine distance), 1.0 mm (C2-C4 distance), 1.7 mm (LYmax), 1.4 mm (LXmax), 1.1 mm (HYmax), 0.9 mm (HXmax), 3.6 % (normLYmax), 4.4 % (normLXmax), 3.9 % (normHYmax), 21.7 mm² (HLarea_min), 6.6 % (normHLarea_min), and 5.3 % (normLYHYmaxDiff).

**Comparing swallows of healthy volunteers vs. patients**

The swallow trials from healthy volunteers and patients differed in PAS scores with patient swallows scoring higher ($U = 97.5$, $z = -3.4$, $p = .001$). Of the 21 healthy volunteer swallows, 15 scored 1 (no penetration or aspiration) on the PAS and 6 scored 2 (penetration above the level of the vocal folds with spontaneous clearance). Of the 21 patient swallows, 8 had
PAS scores of 1 or 2 on the first swallow attempt, 8 had PAS scores of 3, 4 or 5 (penetration with or without clearance), and 5 had a score of 8 (silent penetration and aspiration).

The patients had a longer HLDist $[F(1,40) = 9.9, p = .003]$ at rest. No statistically significant differences were found in C2-C4 distance $[F(1,40) = 8.7, p = .005]$, or larynx to spine distances $[F(1,40) = 0.3, p = .57]$, at rest between healthy volunteers and patients (Fig. 5). Swallows from the two groups differed on raw displacement measures, which were greater in the swallows from the healthy volunteers on LYmax $[F(1,40) = 18.0, p < .0005]$, HYmax $[F(1,40) = 10.9, p = .002]$, and HXmax $[F(1,40) = 70.6, p < .0005]$, but were not statistically significant using an $\alpha = .004$ on LXmax $[F(1,40) = 7.5, p = .009]$, (Fig. 6). The swallows of healthy volunteers had higher percentages on normLYmax $[F(1,40) = 84.7, p < .0005]$ and normHYmax $[F(1,40) = 24.6, p < .0005]$ measures, but did not differ statistically from the patient swallows on normLXmax $[F(1,40) = 8.9, p = .005]$ (Fig. 7). The swallows of healthy volunteers had a smaller vestibule area on normHLarea_min $[F(1,40) = 9.8, p = .003]$, but were not statistically different on HLarea_min $[F(1,40) = 9.0, p = .005]$, (Fig. 8). The measure of the relationship between hyoid and laryngeal elevation [normLYHYmaxDiff (%)] showed less laryngeal elevation in patient swallows $[F(1,40) = 2.6, p < .0005]$, (Fig. 8).

Figure 9 illustrates how normLYmax (%), normLHYmaxDiff (%) and norm HLarea_min (%) may differ between a swallow of a healthy volunteer and a patient. The swallow of the healthy volunteer had a PAS score of 1 (no penetration/aspiration) while the patient swallow PAS score was 3 (penetration without clearance) The swallows had similar normHYmax values of between 75 and 80 % (Fig. 9). However, the patient swallow had laryngeal elevation of 58 % of the resting distance between the hyoid and posterior subglottic air column during vestibule closure, in contrast with the healthy volunteer who had a normLYmax of well over 70% (Fig. 9). The normalized maximum laryngeal elevation of the patient swallow did not overlap with the distribution of normLYmax in healthy volunteers (Fig. 7). The swallows of the patient and healthy volunteer also differed on normalized laryngeal and hyoid elevation
(normLYHYmaxDiff). The patient swallow appeared to have insufficient laryngeal elevation to overcome the extent of hyoid elevation for vestibule closure.

On the discriminant function analysis using displacement measures normalized by differences in anatomy, the linear combination of normLYmax (%), normLXmax (%) and normHYmax (%) significantly discriminated between the swallows of healthy volunteers and patients (Fig. 10) \[\Lambda = 0.29, X^2(3) = 47.0, p < .001, R^2 = .71\], classification accuracy = 93 %, classification accuracy (cross-validated) = 91 %]. The predicted standardized (z) composite score = 1.17*normLYmax_z – 0.36*normHYmax_z – 0.27*normLXmax_z, where the z of each measure was the standardized score. Based on the coefficient associated with normLYmax (1.17), this measure was highly weighted for discriminating between swallows of healthy volunteers and patients. Higher composite z scores occurred in swallows of healthy volunteers (Fig. 10).

PAS scores did not correlate with normHLarea min (r = .25, p = .11) or normLYHYmaxDiff (r = -.21, p = .18). There were nonsignificant trends (\(\alpha = 0.01\)) of association of higher PAS scores with lower normLYmax (r = -.39, p = .011), of higher PAS scores with lower normHYmax (r = -.36, p = .02), and of higher PAS scores with higher normLXmax (r = .36, p = .02).

**Patient swallows with vs. without UES opening**

Of the 21 patient swallows, 12 had UES opening and 9 did not. The PAS scores of swallows with UES opening and those without did not differ (U = 43.5, z = -0.77, p = .44). Of the 12 swallows with UES opening, 4 had PAS scores of 1 or 2, 4 had PAS scores of 3, 4 or 5, and 4 had a score of 8. Of the 9 swallows without UES opening, 4 had PAS scores of 1 or 2, 4 swallows had scores of 3, 4 or 5, and one had a score of 8.

None of the raw displacement, normalized displacement or vestibule closure measures differed between those swallows with UES opening and those without; all p values were greater than .11. Only C2-C4 distance at rest showed a non-significant trend (\(F(1,19) = 4.7, p = .04\) for
the swallows with UES opening to have a mean C2-C4 length of 41.26 mm versus those without opening with a mean of 38.18 mm.

DISCUSSION

This study investigated differences between swallows from healthy volunteers and those from patients on measures of hyolaryngeal displacement, vestibule area and the difference in the extent of laryngeal relative to hyoid elevation normalized by individual differences in anatomy. Measures showing the highest effect sizes were maximum laryngeal elevation normalized by the distance between the hyoid and the posterior-superior subglottic air column at rest (representing the length of the laryngeal vestibule before swallow onset) (Cohen’s $d = 2.9$), and maximum anterior hyoid displacement (Cohen’s $d = 2.7$). Swallows of patients and healthy volunteers also differed in the extent of laryngeal elevation exceeding hyoid elevation, and in the minimum area of the vestibule during swallowing relative to the vestibule area before swallowing. Patient swallows with UES opening did not differ from those without UES opening in penetration/aspiration severity based on PAS scores, or in any of the raw or normalized displacement measures.

The length of the vestibule at rest between the hyoid and the larynx was significantly longer in patients than healthy volunteers (Fig. 5). When laryngeal elevation was corrected for the difference in hyoid to larynx distance at rest, the difference in distribution between the swallows of the healthy volunteers and patients increased on the normalized measure of laryngeal elevation (Cohen’s $d = 2.9$) (Fig. 7) from the distribution of the raw measure of laryngeal elevation (Cohen’s $d = 1.3$) (Fig. 6). Although patients and healthy volunteers did not differ in neck length (Fig. 5), correcting the measure of hyoid elevation for individual differences in neck length (C2 to C4) increased the difference in distribution between swallows of healthy volunteers and patients, from Cohen’s $d = 1.0$ to 1.6 (see HYmax in Fig. 6 and normalized HYmax in Fig.
Thus we found greater differences in distribution between the measures of hyoid and laryngeal elevation when these were corrected for differences in length between the hyoid and larynx at rest and the length of the spine. These findings suggest that the reduction in hyolaryngeal elevation relative to an individual’s anatomical requirement will delineate abnormal swallowing more clearly from normal swallowing.

Between hyoid and laryngeal elevation, we found differences in their ability to distinguish between the swallows of healthy volunteers and patients. There was almost complete separation between the swallows of healthy volunteers and patients based on normalized superior laryngeal displacement (Fig. 7). The extent of laryngeal elevation was much greater than hyoid elevation in healthy volunteers, but patients had similar extents of hyoid and laryngeal elevation (Fig. 6). By measuring the difference between the extent of laryngeal elevation relative to hyoid elevation, almost all patients had a negative difference, meaning that laryngeal elevation was reduced relative to hyoid elevation (Fig. 8). Some patients had hyoid elevation magnitude that exceeded laryngeal elevation magnitude, resulting in more negative values in the difference between laryngeal and hyoid elevation (Fig. 8 and 9). Furthermore, in the linear discriminant function analysis, normalized laryngeal elevation magnitude was weighted most heavily and more than normalized hyoid elevation in discriminating between swallows of healthy volunteers and patients. These results suggest that it may be important to have greater laryngeal elevation than hyoid elevation for swallowing. Insufficient laryngeal elevation during swallowing relative to the length of the individual’s vestibule before the swallow may be more important than inadequate hyoid elevation relative to neck length in characterizing abnormal swallowing. Others have shown that with increasing bolus volumes, hyoid and laryngeal elevation magnitudes were found to increase, but the increase in laryngeal elevation exceeded that of hyoid elevation (3, 6). Similarly, when healthy volunteers produced the Mendelsohn maneuver that required volitional prolongation of hyolaryngeal elevation mid-swallow, laryngeal elevation magnitude exceeded that of hyoid elevation while peak hyoid and laryngeal positions were maintained during the
maneuver (9). Because hyoid and laryngeal elevation occur almost simultaneously at the onset of swallowing (6, 8, 29), an individual may be at a lower risk of material entering the vestibule if laryngeal elevation is greater than hyoid elevation. Depression of the hyoid during swallowing may even reduce penetration/aspiration severity (15). However, when examining the relationship between penetration/aspiration and hyolaryngeal displacements, we found that normalized maximum laryngeal elevation and normalized maximum hyoid elevation had similar inverse correlations with penetration/aspiration severity \((r = -0.39\) and \(-0.36\) respectively) although not statistically significant. As the underlying causes of penetration/aspiration may be multifactorial (16), these correlations suggest that hyolaryngeal elevation may contribute partially to airway protection, in addition to other protective mechanisms such as glottal closure and sensory feedback (7, 17).

We did not find differences between the swallows of patients that had UES opening, and swallows without UES opening on any of the raw or normalized displacement measures. Although anterior hyoid displacement is thought to be important for UES opening (3, 5, 6) it was not significantly different between swallows with and without UES opening. UES relaxation has to occur before the sphincter can open (3, 28), and this may require a patterned response from the brainstem to disinhibit tonic neural firing (30). It is possible that for some patients, the underlying abnormality could be in UES relaxation, which may be independent of impairment in hyolaryngeal displacement. It is also possible that besides maximal displacement magnitudes, other aspects of hyolaryngeal kinematics, such as the timing and velocities of hyolaryngeal movements, may be able to explain the difference in UES opening status in patients.

Our findings have clinical implications on swallowing rehabilitation targeting reduced hyolaryngeal movement magnitudes in dysphagia. It may be more important to focus on improving laryngeal elevation during swallowing than hyoid elevation, as insufficient laryngeal elevation relative to the individual’s vestibule length contributed more to differences between normal and abnormal swallowing than hyoid elevation. Having hyoid elevation that exceeds
laryngeal elevation may be counter-productive to vestibule closure in patients with dysphagia (Fig. 8 and 9). Based on data using spatial normalized measures (Fig. 7), the optimal maximum superior laryngeal displacement should be at least 70% of the individual’s distance between the hyoid and the posterior larynx at rest, instead of a specific magnitude in mm or cm. This might serve as a target as well as an outcome measure in swallowing rehabilitation addressing laryngeal elevation and approximation between the larynx and the hyoid, such as the Mendelsohn maneuver (9), the Shaker exercise (18) and intramuscular stimulation (2). Future studies could examine if normalized maximum laryngeal elevation magnitude would be sensitive to changes in swallowing function associated with the recovery of swallowing with treatment or deterioration due to disease progression.

A limitation in this study was the dichotomous classification of UES opening status as 2 discrete groups of swallows, obfuscating different degrees of bolus clearance through the sphincter. The extent of UES opening during swallowing as a continuous variable might be better related to measures of hyolaryngeal displacement. The healthy volunteers were on average younger than the patients. However, no clear trends of the effect of age on hyolaryngeal displacement magnitudes were identified across different studies (19). The patients in this study were concurrently participating in other dysphagia treatment research protocols, which tend to attract more severely impaired patients who did not benefit from conventional therapy. Likely patients with milder forms of dysphagia might have been under-represented in this sample, and the group differences in this study might be more optimistic than in typical patient populations with a more distributed range of dysphagia severities.

In summary, we found that swallows with and without UES opening did not differ in hyolaryngeal displacements. However, spatially normalized measures of hyolaryngeal elevation showed greater differences between normal and abnormal swallowing than raw measures. Normalized measures of laryngeal vestibule area during swallowing, the difference between maximum laryngeal and hyoid elevation, and the raw measure of maximum anterior hyoid
displacement also differed between normal and abnormal swallows. Between hyoid and laryngeal elevation, the latter contributed more to differences between swallows of healthy volunteers and patients. Having insufficient laryngeal elevation may be more detrimental to swallowing than insufficient hyoid elevation in dysphagia.

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REFERENCES


FIGURE LEGENDS

Figure 1.
Left lateral view of a fluoroscopic video frame. The $y$-axis intersects the anterior-inferior corners of the 2nd (C2) and 4th (C4) cervical vertebrae. The $x$-axis is at $90^\circ$ to $y$ and intersects the anterior-inferior corner of C4. Hyoid (anterior-inferior corner of the hyoid bone) and larynx (anterior-superior corner of the subglottic air column) positions are tracked in the $x$ and $y$ dimensions during swallowing. The hyoid bone and the superior aspect of the subglottic air column are outlined.

Figure 2.
Left lateral view of a fluoroscopic video frame. The numbers indicate measurement points: 1) anterior-inferior corner of C2; 2) anterior-inferior corner of C4; 3) anterior-inferior corner of the hyoid bone; 4) anterior-superior corner of the subglottic air column; and, 5) posterior-superior corner of the subglottic air column.

The letters indicate anatomical distance and area measures obtained from the first frame that was tracked in each video. A) HLDist: Distance between anterior-inferior corner of the hyoid bone (3) and posterior-superior corner of the subglottic air column (5); B) Larynx to spine distance: Horizontal distance between the anterior-superior corner of the subglottic air column (4) and the $y$ axis; C) C2-C4 distance: Distance between the anterior-inferior corners of C2 and C4; and, D) HLarea: Area of the triangle bound by points (3), (4) and (5).

Figure 3.
Superior laryngeal displacement across time in a subject, where displacement at the first data point = 0. Maximum superior laryngeal displacement (LYmax) is the difference between the
maximum y and initial positions. Line (a) is the time when the bolus head reached the angle of the mandible. Line (b) represents the time when the tail of the bolus passed the level of the 6th cervical vertebra (C6). Motion tracking began 1 s before time (a) and ended 1 s after time (b).

Figure 4.

Maximum superior laryngeal displacement (LYmax) measured from the displacement time series of a patient with 2 swallow attempts and no bolus passage through the UES. The hyoid and larynx were in stable resting positions 1 s before the bolus head reached the angle of the mandible, hence motion tracking began at this point. Increasing displacement indicates upward motion, decreasing displacement indicates downward motion.

Figure 5.

Comparisons between healthy volunteers (HV) (left boxplot) and patients (Pts) (right boxplot) on anatomical measures in mm of, *Left:* Distance between the hyoid and the posterior-superior subglottic air column (HLdist); *Middle:* C2 to C4 distance; *Right:* Distance between the larynx and the cervical spine at rest.

Figure 6.

Comparison of raw maximum displacements in mm during swallowing between healthy volunteers (HV) (left boxplot) and patients (Pts) (right boxplot), on, *Left:* Superior laryngeal (LYmax); *Middle:* Anterior laryngeal LXmax, superior hyoid HYmax; *Right:* Anterior hyoid HXmax movement during swallowing.

Figure 7.

Comparison of normalized maximum displacements (%) during swallowing between healthy volunteers (HV) (left boxplot) and patients (Pts) (right boxplot) on measures of, *Left:* Superior
laryngeal displacement normalized as % of distance between the hyoid and the posterior-superior subglottic air column (normLYmax); Middle: Anterior laryngeal displacement normalized as % of the larynx to spine distance (normLXmax), and Right: Superior hyoid displacement normalized as % of C2 to C4 distance (normHYmax).

Figure 8.
Comparison of measures of vestibule closure and percent difference in larynx to hyoid elevation during swallowing in healthy volunteers (left boxplot) and patients (right boxplot). The measures are, Left: Minimum area of the vestibule (HLarea_min) in mm²; Middle: Minimum area of the vestibule during swallowing as % of the vestibule area at rest (normHLarea_min); and Right: Difference between normalized maximum superior laryngeal displacement and normalized maximum superior hyoid displacement in % (normLYHYmaxDiff).

Figure 9.
Top: Time series of normalized movement trajectories of hyoid (normHyoidY, %; dotted line) and laryngeal elevation (normLarynxY, %; thin solid line) and % change in laryngeal vestibule area (normVestibule Area; thick solid line) during swallowing of a healthy individual with no penetration or aspiration (PAS = 1).
Bottom: The corresponding time series of a patient’s swallow that had penetration above the level of the vocal folds (PAS score = 3), with no vestibule closure seen during swallowing in the fluoroscopic recording.
Arrows indicate the magnitude (on y-axis) of normalized maximum superior hyoid displacement (normHYmax, %), normalized maximum superior laryngeal displacement (normLYmax, %), and normalized minimum area of the vestibule (normHLarea_min, %). Double pointed arrows indicate the difference between normLYmax and normHYmax (normLYHYmaxDiff) in the
patient’s swallow. normHyoidY and normLarynxY were not at 0 % at time = 0, as the formulae for calculating their normalized values incorporated a constant (see text for formula).

Figure 10.
Distributions of composite z scores of swallows from patients (N=21; left distribution) and those from healthy volunteers (N=21; right distribution) using normalized displacement measures. The y-axis represents number of swallows, the x-axis represents composite z scores (see text for z score equation). Bars in the darkest shade indicate overlaps between the swallows from the 2 groups.
FIGURES

Figure 1.
Figure 3.
Figure 4.

- Bolus head reaches angle of mandible
- Lowest position after peak displacement
- LYmax

1st swallow attempt
2nd swallow attempt
Figure 5.

Hyoid to posterior larynx distance (HLdist)

C2 to C4 distance

Larynx to spine distance

F=9.90, p=.003

ns

ns
Figure 6.

Larynx Y displacement (LY\text{max})

F=18.1, p<.0005

Larynx X displacement (LX\text{max})

ns

Hyoid Y displacement (HY\text{max})

F=10.9, p=.002

Hyoid X displacement (HX\text{max})

F=70.6, p<.0005
Figure 7.

Normalized Larynx Y displacement (normLYmax)

Normalized Larynx X displacement (normLXmax)

Normalized Hyoid Y displacement (normHYmax)

F=84.7, p<.0005

ns

F=24.6, p<.0005
Figure 8.

- **Minimum Vestibule Area (HLarea_min)**

- **Percent of Vestibule Area (normHLarea_min)**

- **normLYmax − normHYmax (normLYHYmaxDiff)**

  - ns
  - $F=9.8, p=.003$
  - $F=26.9, p<.0005$
Figure 9.

Displacement or area (%)

Healthy volunteer

- normHyoid Y
- normLarynx Y
- normVestibule area

Patient

- normHyoid Y
- normLarynx Y
- normVestibule area

normHLarea_min

normLYHYmax Diff = -18%

normHYmax

normLYmax

normHY max

normLY max

normHLarea_min

Time (s)
Figure 10.

Frequency count

Patients
- Smaller normLYmax
- Larger normHYmax
- Larger normLXmax

Healthy volunteers
- Larger normLYmax
- Smaller normHYmax
- Smaller normLXmax
TITLE
Differentiating normal and abnormal swallowing using temporal measures of laryngeal elevation

RUNNING HEAD
Temporal measures of laryngeal elevation
ABSTRACT

Laryngeal vestibule closure and upper esophageal sphincter opening (UES) are important for safe swallowing. The extent of laryngeal elevation relative to the size of the laryngeal vestibule may determine the extent of vestibule closure. On the other hand, excessive hyoid elevation during swallowing relative to laryngeal elevation may reduce swallow safety by increasing laryngeal vestibule size. We investigated if measures of laryngeal elevation peak velocity, timing and movement patterning would differ between patients and controls swallowing more than corresponding measures of hyoid elevation. Single swallows recorded from videofluoroscopy from 21 healthy volunteers and 21 patients were analyzed using 2D motion analysis of hyoid and laryngeal movements in the anterior and superior directions. Hyolaryngeal peak velocity magnitudes, time to peak velocities, and the number of zero crossings in movement velocity over time were measured. Normal and disordered swallows differed on timing, patterning and peak velocity magnitudes of laryngeal elevation and anterior hyoid movement, but not on hyoid elevation peak velocity magnitude. Reduced laryngeal elevation peak velocity and reduced movement smoothness correlated with penetration/aspiration severity. In normal swallows, the time of the peak vestibule closure velocity correlated with that of the peak velocity of laryngeal elevation, but not with the time of the peak velocity of hyoid elevation. Patient swallows did not show coordination between the time of peak vestibule closure velocity and the time of peak velocity of any hyolaryngeal movement. Upward laryngeal motion and anterior hyoid motion may be the most crucial elements of hyolaryngeal movement for safe swallowing.

KEYWORDS

Deglutition, dysphagia, hyoid, larynx, temporal measure, movement pattern
INTRODUCTION

Laryngeal vestibule closure and upper esophageal sphincter (UES) opening are important in the pharyngeal phase of swallowing to ensure safe and efficient swallows. Movements of the hyoid bone and the larynx are thought to contribute to laryngeal vestibule closure and UES opening (5-7, 10, 14, 22, 50, 51). Previously, we found that the swallows of patients with dysphagia had insufficient laryngeal elevation magnitudes relative to the extent of hyoid elevation required for laryngeal vestibule closure (Wong et al., in preparation). This suggests that the extent of laryngeal elevation may be more important than that of hyoid elevation for laryngeal vestibule closure for safe swallowing.

Displacement magnitudes do not fully characterize the swallowing motor response. Temporal relationships among hyoid and laryngeal movements are also important for determining the integrity of swallow patterning and coordination, as has been shown for gait (20). Collectively, spatial and temporal measures allow greater specificity in diagnosing swallowing impairment and setting treatment targets for swallowing rehabilitation.

Compared to normal swallows, disordered swallows may be delayed in hyolaryngeal movement onset and the times when their most rapid movements occur (2, 13, 16, 18, 19, 38). This may indicate slower swallowing motor response in dysphagia. Some have attributed penetration/aspiration severity to either a delayed initiation or reduced speed of laryngeal elevation (2, 13, 38), while others have placed greater importance on the timing of hyoid motion (16, 18). Patients also have reduced velocity of hyolaryngeal movement during swallowing compared to healthy individuals (13, 19, 49). As a greater extent of laryngeal elevation may be more important than hyoid elevation for safe swallowing (Wong et al., in preparation), we hypothesized that measures of timing and velocity of laryngeal elevation would differ between normal and abnormal swallowing more than corresponding measures of hyoid elevation.

A limitation in measuring the timing of an event of interest (e.g. time of occurrence of peak velocity) relative to a referent swallow event (e.g. onset of swallowing) is that this does not
inform about movement patterning. Movement pattern analysis is relevant to swallowing, as swallowing involves a patterned response in the brainstem (11, 12, 17, 31), in addition to cortical input for initiation, modulation and sensory integration (25-28, 33, 35, 44). In skilled motor tasks, healthy individuals may vary in the overall movement duration and the onset of movement initiation. However, the movement pattern may be consistent once the time at which a movement event occurs is normalized by the total movement duration. This is evident in speech motor control (42, 43), locomotion (3, 9, 36, 41) and tongue movement during swallowing (45).

Besides normalizing event time by total movement duration, another way to quantify movement patterning is to count the number of peaks in the velocity over time for the movement of interest (37). Continuous and smooth motion in one direction has a bell-shaped velocity peak (1, 8), whereas multiple velocity peaks during motion may indicate reduced movement smoothness (4, 39). Based on this principle, when the larynx elevates and descends during swallowing, its velocity-time curve should have a positive peak at the time of most rapid elevation, followed by a negative peak at the time of most rapid descent, and likewise as it moves along the anterior-posterior plane. When using the number of velocity peaks to quantify hyolaryngeal movement smoothness in swallowing, a problem may arise when patients with severe dysphagia have a limited range of motion in the hyoid and larynx, and velocity peaks are dampened. For example, using a threshold of 20 mm/s, no velocity peaks of hyoid motion could be identified in some patients with dysphagia who might have limited hyoid movement, while other patients showed more velocity peaks than healthy controls (37). The results of peak analysis may therefore vary depending on the threshold set by the investigator in defining a velocity peak. Less number of peaks identified above a threshold velocity may be an indication of limited range of motion rather than a measure of greater movement smoothness. We proposed instead to measure the number of zero crossings in the velocity-time graphs of hyolaryngeal movements to examine movement patterns. This could be measured even in patients with very limited hyolaryngeal motion, as it did not require movement velocity to exceed an arbitrary threshold.
In this study, we examined whether swallows of healthy participants and patients would differ in maximum velocities of hyolaryngeal movements and maximum rate of reduction in the area of the vestibule. In addition, we compared between healthy individuals and patients in the time of occurrence of peak hyolaryngeal movement velocities relative to the time of initial swallow movement, as well as in the patterning of hyolaryngeal movements. We measured movement patterning in 2 ways, by normalizing the time of occurrence of peak hyolaryngeal velocities relative to the total swallow duration, and by counting the number of zero crossings in the velocity time series of hyolaryngeal movements. We hypothesized that patients would exhibit reduced magnitudes and delayed occurrences of peak hyolaryngeal velocities. As UES opening may depend on timely coordination among hyolaryngeal movements (5, 10, 14, 22, 50, 51), we also compared between swallows of patients, to determine if failure to open the UES was related to abnormal swallow patterning in addition to delay in time to peak hyolaryngeal movement velocities.

**MATERIALS and METHODS**

**Subjects**

Adults between 20 and 80 years of age were recruited as healthy volunteers, and gave informed consent to participate in a protocol approved by the Institutional Review Boards (IRBs) at James Madison University and Sentara Rockingham Memorial Hospital Medical Center. Volunteers were excluded if they reported: swallowing difficulty, history of neurological disorder affecting swallowing function, acid reflux diagnosed by a physician, and history of head and neck cancer. De-identified archived video recordings were also gathered from healthy subjects and patients with dysphagia under IRB approved archival protocols from the National Institute of Neurological Disorders and Stroke. The patients had dysphagia following either neurological impairment or head and neck cancer. Additional patients were recruited from the Voice and
Swallow Services at Sentara Rockingham Memorial Hospital Medical Center and swallowing studies at James Madison University. They participated after informed consent. They were included if they were above the age of 12, and had dysphagia either following treatment for head and neck cancer or neurological disorders such as stroke. There were no restrictions on the time since onset of their medical diagnosis or dysphagia. All of these videofluoroscopy recordings were reported on in an earlier study of the extent of hyolaryngeal displacement in healthy volunteers and patients with dysphagia (Wong et al., in preparation).

Procedure

A radio-opaque ball with a 19 mm diameter was taped to the side of the subject’s neck posterior to the spine to be used for converting pixels into millimeters. A digital Siemens fluoroscope (Model AXIOM Luminos TF) was set up for a lateral view from anterior neck extending inferiorly from the trachea and below the upper esophageal sphincter, to posterior spine from C1 to C6 and extending superiorly to the floor of the nasal cavity (Fig. 1). Five ml of thin liquid barium (Varibar®, 40% weight/volume) was delivered orally by the examiner by spoon or syringe. The participant was instructed to hold the liquid in the mouth until the fluoroscope was then turned on and the examiner gave the command to “swallow now”. Magnification was unchanged throughout the swallow. Each fluoroscopic swallow trial was captured at 30 frames/s and saved in .avi format using a D-scope® System (D-scope® Systems, Brooklyn, NY).

Data processing

Videofluoroscopic recordings were imported into Peak Motus 8.5 (Vicon Denver, Centennial, CO) for distance calibration and two-dimensional motion analysis. One swallow trial per subject was analyzed.
**Conversion into millimeters:** To convert pixels into millimeters, the diameter of the calibration ball was measured on a video frame in the recording. For consistency in frame selection across videos, we used the frame when the head of the bolus reached the angle of the mandible. If motion blur occurred on this frame due to subject movement, then another video frame with adequate clarity and contrast of the calibration ball within the swallow sequence was selected. As magnification was unchanged during fluoroscopy within a swallow trial, the same scaling factor was automatically applied to other frames in the same recording.

**Spatial analysis:** The anterior-inferior corner of C4 served as the origin for the x and y-axes in the horizontal and vertical dimensions respectively (Fig. 1). The y-axis connected the origin to the anterior-inferior corner of C2, while the x-axis was perpendicular to the y-axis at the origin (Fig. 1). A spatial model of the measurement points for motion analysis was set up in Peak Motus 8.5 to manually track the position of each point frame by frame using a cursor. These points were (Fig. 1): 1) Anterior-inferior corner of C2; 2) Anterior-inferior corner of C4; 3) Anterior-inferior corner of the hyoid bone; 4) Anterior-superior corner of the subglottic air column to track the larynx; and, 5) Posterior-superior corner of the subglottic air column. Three distances between points (3), (4) and (5) were also measured (Fig. 1).

**Time periods measured:** Measurement for each swallow started on the frame that was 1s before the head of the bolus reached the angle of the mandible. However, if the hyoid and larynx had already begun movement at this time point, then motion analysis was begun further back in time closer to the start of the fluoroscopic recording to capture the resting positions of the hyoid and larynx while the bolus was held in the oral cavity. For healthy volunteers, motion tracking continued until 1s after the tail of the bolus passed the anterior-inferior corner of C6. However, many of the patients swallowed multiple times per bolus and some had insufficient UES opening for the bolus to flow past the level of C6. Therefore, motion tracking of the swallow trials of all patients continued until any of the following events occurred: onset of a second swallow,
cessation of fluoroscopy, or when excessive motion blur accompanied patient movement (e.g. during coughing).

**Filtering the kinematic time series data:** A fourth-order zero-phase Butterworth low-pass filter with a cutoff frequency of 2 Hz was applied within Peak Motus 8.5 to smooth the time series positional data of the hyoid and the larynx for x and y over time. As recursive forward and backward passes were made in the filter process, no time lag was expected in the filtered data. From the smoothed hyoid and laryngeal positional data, velocity time series data for x and y over time, and segmental distance time series data were then derived within Peak Motus 8.5 without further smoothing. The position, segmental distance and velocity time series data were exported into Matlab R2013a (The Mathworks, Inc., Natick, MA). Each time series had a temporal resolution of 30 data points per second.

**Velocity and timing measures from kinematic data:** The initial x and y positions of the hyoid and larynx were linearly transposed so that all initial positions (i.e. the first data point) had a displacement of 0 mm (Fig. 2). This produced 4 displacement time series: anterior hyoid (HX), superior hyoid (HY), anterior larynx (LX) and superior larynx (LY), and 4 corresponding velocity time series (HXvel, HYvel, LXvel, LYvel). Based on these data, swallow onset and swallow offset times were defined as follows.

\[
\text{Swallow onset (ms)} = \text{Time of earliest movement of either the hyoid or larynx in anterior, posterior, inferior, or superior direction.}
\]

In each of HXvel, HYvel, LXvel and LYvel, we identified the first zero crossing in either the positive or negative direction that led to an increase in velocity to more than 10% of the maximum positive velocity (Fig. 2). The earliest time amongst them was defined as the time of swallow onset (Fig. 2). The 10% threshold was chosen, so that small fluctuations in velocity just above or below 0 mm/s would not be identified as a movement onset time for swallowing. We did not define swallow onset as the time when the bolus crosses the ramus of the mandible (18, 19, 23, 32), the onset of superior hyoid motion (37), or the onset of superior laryngeal elevation (2, 23, 24). This was because healthy individuals
might vary in when they initiate swallowing; some only after the bolus had travelled deep into the pharynx (29, 46), hence bolus location may not be a reliable marker of swallow onset. Secondly, although healthy individuals may produce hyoid or laryngeal elevation as the first event in the pharyngeal swallow (30), some variability may still exist (15, 23). Our pilot data also suggested that disordered swallows might deviate from normal movement initiation. Hence our definition of swallow onset captured the earliest movement of either the hyoid or larynx in any direction.

Swallow offset (ms) = Time when the larynx reached its lowest y position after peak elevation in the first swallow, based on the LY displacement time series (Fig. 2).

Swallow duration (ms) = Swallow offset – swallow onset

Based on these definitions, the following measures were derived.

Measures of movement velocity:

1. Peak velocities of hyolaryngeal movements (mm/s). These were the maximum positive velocities for anterior hyoid (HXvelmax), superior hyoid (HYvelmax), anterior laryngeal (LXvelmax) and superior laryngeal (LYvelmax) movements (Fig. 2). The difference between LYvelmax and HYvelmax was also derived (LYHYvelDiff). This represented the difference in the peak laryngeal upward velocity and the peak hyoid upward velocity during swallowing.

2. Peak negative velocity in area between hyoid and larynx (mm$^2$/s) (HLarea_redmax). This represented the greatest negative peak velocity of vestibule closure between the hyoid and larynx during swallowing. The area of the vestibule was calculated based on the area of the triangle bound by the segmental distances connecting the anterior-inferior corner of the hyoid, the anterior-superior subglottic air column, and the posterior-superior subglottic air column (Fig. 1), using Heron’s formula (48). This area was computed over time from swallow onset to offset, which produced a vestibule area time series. The velocity of vestibule area at each time point (at intervals of 1/30 s) was computed using a 2-point central difference formula:
\begin{equation*}
Rate\ of\ change\ at\ time_n (\text{mm}^2/\text{s}) = \frac{\text{vestibule\ area\ at\ time}_{n+1}-\text{vestibule\ area\ at\ time}_{n-1}}{2/30}
\end{equation*}

At the first time point,
\begin{equation*}
Rate\ of\ change\ (\text{mm}^2/\text{s}) = \frac{\text{vestibule\ area\ at\ time\ point}_2-\text{vestibule\ area\ at\ time\ point}_1}{1/30}
\end{equation*}

At the last time point \(N\),
\begin{equation*}
Rate\ of\ change\ (\text{mm}^2/\text{s}) = \frac{\text{vestibule\ area\ at\ time\ point}_N-\text{vestibule\ area\ at\ time\ point}_{N-1}}{1/30}
\end{equation*}

The magnitude of the most negative velocity in area change was identified as \(\text{HLarea\_redmax\ (mm}^2/\text{s)}\).

**Measures of movement delay:**

1. **Time of maximum hyolaryngeal movement velocity in ms.** This represented the time of occurrence of most rapid hyolaryngeal movements relative to swallow onset. In each of \(\text{HXvel}, \text{HYvel}, \text{LXvel}\) and \(\text{LYvel}\), we identified the time when maximum positive velocity occurred (Fig. 2). The swallow onset was subtracted from each of these 4 time points to produce the time of peak velocity for anterior hyoid (\(\text{HXvel\_ms}\)), superior hyoid (\(\text{HYvel\_ms}\)), anterior laryngeal (\(\text{LXvel\_ms}\)) and superior laryngeal (\(\text{LYvel\_ms}\)) motion.

2. **Time of maximum velocity in area reduction between the hyoid and larynx in ms** (\(\text{HLarea\_rapid\_ms}\)). This represented the time of occurrence of the negative peak in vestibule area relative to swallow onset. The time at which \(\text{HLarea\_redmax\ (mm}^2/\text{s)}\) occurred was identified and swallow onset was subtracted from it.

**Measures of movement patterning:**

1. **Normalized times of hyolaryngeal peak velocity (%)**. The time of hyolaryngeal peak velocities (\(\text{HXvel\_ms}, \text{HYvel\_ms}, \text{LXvel\_ms}\) and \(\text{LYvel\_ms}\)) were computed as percentages of the swallow duration rather than raw time. They represent patterning of rapid hyolaryngeal movements within the swallow cycle. Anterior hyoid (\(\text{HXvel\_\%}\)), superior hyoid
(HYvel_%), anterior laryngeal (LXvel_%) and superior laryngeal (LYvel_%) normalized
time of peak velocity relative to the swallow duration were computed using the formula,

\[
\text{normalized time of peak velocity} \ (\%) = \frac{HXvel\ _ms, HYvel\ _ms, LXvel\ _ms, or LYvel\ _ms}{\text{swallow offset (ms)} - \text{swallow onset (ms)}} \times 100\ \%.
\]

2. **Normalized time of maximum rate of reduction in area between hyoid and larynx**

(\%) (HLarea_rapid, %). This represented the % time when most rapid reduction in the
vestibule area between the hyoid and larynx occurred, relative to swallow duration:

\[
HLarea\_rapid \ (%) = \frac{HLarea\_rapid (ms)}{\text{swallow offset (ms)} - \text{swallow onset (ms)}} \times 100\ \%.
\]

3. **Number of zero crossings in the velocity time series.** This represented hyolaryngeal
movement patterning (smoothness) during swallowing. More repeated forward and
backward, or up and down movements during swallowing would produce more zero
crossings in the velocity time series of hyoid and laryngeal movements (Fig. 3). From the
velocity time series of HXvel, HYvel, LXvel, and LYvel between swallow onset and offset,
the number of zero crossings were derived for anterior hyoid (HXzerocross), superior hyoid
(HYzerocross), anterior laryngeal (LXzerocross) and superior laryngeal (LYzerocross)
movement.

**Statistical analyses**

**Measurement reliability:** The same investigator replicated motion analysis of the swallow trials
from the healthy volunteers and patients. Intra-class correlation coefficients (ICCs) were
computed based on a two-way random effects model (assuming the effects of subject and
swallow trial were random), for the following 20 measures of movement velocity, timing and
movement patterning: 1) HXvelmax, 2) HYvelmax, 3) LXvelmax, 4) LYvelmax, 5)
LYHYvelDiff, 6) HLarea_redmax, 7) HXvel_ms, 8) HYvel_ms, 9) LXvel_ms, 10) LYvel_ms,
11) HLarea_rapid_ms, 12) HXvel_%, 13) HYvel_%, 14) LXvel_%, 15) LYvel_%, 16)
The absolute measurement error (absolute difference between the first and second measure) between the first and replicated data sets was also computed for each measure.

**Comparisons between swallows of healthy volunteers and patients with dysphagia:** Each of the swallows produced by healthy volunteers and by patients was rated on the Penetration-Aspiration Scale (PAS) (40) to measure the integrity of airway protection.

One-way analyses of variance (ANOVAs) were conducted to determine if healthy volunteers and patients differed on the following 20 measures of movement velocity, delay and movement patterning: 1) HXvelmax, 2) HYvelmax, 3) LXvelmax, 4) LYvelmax, 5) LYHYvelDiff, 6) HLarea_redmax, 7) HXvel_ms, 8) HYvel_ms, 9) LXvel_ms, 10) LYvel_ms, 11) HLarea_rapid_ms, 12) HXvel_, 13) HYvel_, 14) LXvel_, 15) LYvel_, 16) HLarea_rapid_, 17) HXzerocross, 18) HYzerocross, 19) LXzerocross, 20) LYzerocross.

Statistical significance was set at $\alpha = .0025$ to account for multiple comparisons.

**Comparisons between patient swallows:** In a previous study (Wong et al., in preparation), swallows produced by patients that were classified as “without UES opening” did not have any bolus passing through the UES. Those that had some of the bolus, even if it was a small amount, passing through the UES were classified as swallows “with UES opening”.

To determine if there were differences between the swallows of patients with UES opening and without UES opening, ANOVAs were conducted on the same 20 measures as above, using a Bonferroni-corrected $\alpha = .0025$ to account for multiple analyses.

Statistical analyses were conducted using IBM SPSS Statistics for Macintosh, Version 22.0 (IBM Corp, Armonk, NY).
RESULTS

Subject characteristics

Twenty-one healthy adults (9 males) between the ages of 20 and 69 years (mean = 39) participated as healthy volunteers. They were also participants in 2 other studies reported in separate papers (Wong et al. a, in preparation, Wong et al. b, in preparation). The swallows of the healthy volunteers did not evidence dysphagia based on Penetration-Aspiration Scale scores and the investigator’s clinical judgment on viewing the videofluoroscopic recordings.

Twenty-one patients were included in this study (16 males, mean age = 64 years, range: 20 to 82 years old). They were also participants in a separate study on displacement measures of hyolaryngeal movement reported in a separate paper (Wong et al. b, in preparation), and had medical diagnoses of neurological disorders (9 subjects) or head and neck cancer (12 subjects). Patients’ swallows evidenced dysphagia based on Penetration-Aspiration Scale scores, and the investigator’s clinical judgment on viewing the videofluoroscopic recordings.

Measurement reliability

Measures of 42 swallow trials (21 healthy volunteers, 21 patients) were replicated. Absolute measurement errors for the 20 measures were as follows. HXvelmax: 3.1 mm/s; HYvelmax: 3.3 mm/s; LXvelmax: 3.8 mm/s; LYvelmax: 6.0 mm/s; LYHYvelDiff: 5.6 mm/s; HLarea_redmax: 101 mm²/s; HXvel_ms: 277 ms; HYvel_ms: 196 ms; LXvel_ms: 322 ms; LYvel_ms: 333 ms; HLarea_rapid_ms: 356 ms; HXvel_%: 7.5 %; HYvel_%: 6.1 %; LXvel_%: 9.7 %; LYvel_%: 9.4 %; HLarea_rapid_%: 11.6 %; HXzerocross: 1.2; HYzerocross: 1.3; LXzerocross: 1.9; LYzerocross: 1.3. ICC coefficients for the 20 measures were between .48 (LYvel_ms) and .99 (HXvel). Single-measures ICC coefficients were lower, between .31(LYvel_ms) and .98 (HXvel). The averaged measures derived from the first and replicated datasets were used for each subject in subsequent statistical analyses.
Comparing swallows of healthy volunteers vs. patients

As was previously reported (Wong et al. b, in preparation), PAS scores differed between the swallows of healthy volunteers and patients, with patient swallows scoring higher ($U = 97.5$, $z = -3.4$, $p = .001$, $r = -.52$).

Compared to healthy volunteers, the swallows of patients had lower HXvelmax ($F(1,40) = 75.4$, $p < .001$), LXvelmax ($F(1,40) = 11.6$, $p = .001$), LYvelmax ($F(1,40) = 31.2$, $p < .001$), and LYHYvelDiff ($F(1,40) = 26.3$, $p < .001$), but HYvelmax was not significantly different between groups ($F(1,40) = 3.6$, $p = .007$), (Fig. 4). Healthy volunteers had higher LYvelmax than HYvelmax; and LYHYvelDiff was greater than 0 mm/s on all of their swallows (Fig. 4). On the other hand, some patients had negative LYHYvelDiff (Fig. 4). HL_area_redmax did not differ between patients and healthy volunteers ($F(1,40) = 2.8$, $p = .10$).

A linear discriminant function analysis was conducted to determine whether velocity magnitudes could differentiate between the swallows of healthy volunteers and patients. The data met the assumption of homogeneity of covariance matrices (Box’s $M = 16.5$, $p = .14$). A discriminant function with HXvelmax, HYvelmax, LXvelmax and LYvelmax significantly differentiated between the swallows of the 2 groups, $\Lambda = 0.30$, $X^2(4) = 45.6$, $p < .001$, $R_c^2 = .84$. The predicted standardized ($z$) composite score = $0.84 \times$ HXvelmax$_z - 0.05 \times$ HYvelmax$_z - 0.09 \times$ LXvelmax$_z + 0.49 \times$ LYvelmax$_z$, where the $z$ of each measure was the standardized score. The coefficients associated with HXvelmax (0.84) and LYvelmax (0.49) were weighted more heavily for discriminating between the swallows of healthy volunteers (higher composite z scores) and patients (lower composite z scores), while HYvelmax and LXvelmax had negligible contributions.

The swallows of patients had later peak velocities than the healthy volunteers in HXvel_ms ($F(1,40) = 19.4$, $p < .001$), HYvel_ms ($F(1,40) = 12.7$, $p = .001$), LXvel_ms ($F(1,40) = 13.6$, $p = .001$), and LYvel_ms ($F(1,40) = 10.9$, $p = .002$), (Fig. 5). The time at which
maximum reduction in vestibule area occurred (HLarea_rapid_ms) did not differ between the healthy volunteers and patients ($F (1,40) = 3.8, p = .06$).

To examine if hyolaryngeal peak velocity time might relate to the time of peak closing velocity in the vestibule area in normal swallowing, HLarea_rapid_ms was correlated separately with HXvel_ms, HYvel_ms, LXvel_ms and LYvel_ms in the swallows of the 21 healthy volunteers ($\alpha = .0125$). The same relationships were examined in the swallows of the 21 patients ($\alpha = .0125$). In swallows of healthy volunteers, HLarea_rapid_ms correlated significantly with HXvel_ms ($r = .87, p < .001$), LXvel_ms ($r = .80, p < .001$) and LYvel_ms ($r = .89, p < .001$), but not with HYvel_ms ($r = .36, p = .11$) (Fig. 6). In patient swallows, none of the occurrences of peak hyolaryngeal velocities correlated with HLarea_rapid_ms (HXvel_ms: $r = .04, p = .85$; HYvel_ms: $r = -.06, p = .78$; LXvel_ms: $r = .09, p = .70$; LYvel_ms: $r = .12, p = .58$), (Fig. 6).

Fig. 7 illustrates these relationships in a healthy individual’s swallow. The most rapid decrease in laryngeal vestibule area occurred shortly after peak velocities were achieved in laryngeal elevation and anterior hyoid movement; the reverse was observed at the end of the swallow during vestibule opening and laryngeal descent.

Patient swallows had more zero crossings in the velocity time series of HX ($F (1,40) = 21.1, p < .001$), HY ($F (1,40) = 20.7, p < .001$) and LY movements ($F (1,40) = 32.4, p < .001$), but they did not differ from healthy volunteers on LXzerocross ($F (1,40) = 8.3, p = .006$), (Fig. 8).

Patient swallows had peak velocities occurring later in the normalized swallow cycle than the swallows of healthy volunteers on the measures of HXvel_% ($F (1,40) = 16.5, p < .001$), HYvel_% ($F (1,40) = 12.5, p = .001$), and LXvel_% ($F (1,40) = 15.9, p < .001$). LYvel_% did not differ between the swallows of the patients and healthy volunteers ($F (1,40) = 6.4, p = .02$) using $\alpha = .0025$ (Fig. 9).

The measures that differed between swallows of patients and healthy volunteers (HXvelmax, LYvelmax, LYHYvelDiff and LYzerocross) were examined for relationships with
penetration/aspiration severity on the PAS, using a corrected $\alpha$ of .0125. Higher PAS scores (i.e. greater penetration/aspiration severity) were associated with lower LYvelmax ($r = -.484, p = .001$), lower LYHYvelDiff ($r = -.384, p = .012$) and higher LYzerocross ($r = .395, p = .01$). There was a non-significant trend of association of higher PAS scores with lower HXvelmax ($r = -.379, p = .013$).

**Patient swallows with vs. without UES opening**

Of the 21 swallow trials from patients, 12 had UES opening and 9 did not. Nine swallows came from patients with neurological diagnoses (3 had UES opening) and 12 were from patients with head and neck cancer (9 had UES opening). As was previously reported (Wong et al. b, in preparation), there was no significant difference in PAS scores between the swallows of patients with UES opening and those without ($U = 43.5, z = -0.77, p = .44, r = -.16$).

None of the 20 measures of velocity magnitudes, timing of peak velocities or number of zero crossings differed between patient swallows with vs. without UES opening; all $p$ values were greater than .12.

**DISCUSSION**

We investigated differences between swallows of patients and healthy volunteers on measures of velocity magnitudes, timing and patterning of hyolaryngeal movements. Patient swallows were characterized by lower maximum velocities of anterior hyoid movement and laryngeal elevation, and more recursive up and down movements of the larynx during swallowing. In patient swallows, the time of peak closing velocity between the hyoid and larynx during swallowing was unrelated to the peak hyolaryngeal velocity times. On the other hand, in the swallows of healthy volunteers, the time of peak vestibule closing velocity was highly correlated with the time of laryngeal elevation peak velocity. More severe penetration/aspiration
was associated with reduced laryngeal elevation peak velocity relative to hyoid elevation velocity, and less smooth laryngeal elevation movement. Patient swallows with vs. without UES opening did not differ in any of the timing, patterning or peak velocity measures.

Swallows produced by patients exhibited hyolaryngeal movements that were slower, more delayed, and less smooth. Several measures showed large differences between the swallows of patients and healthy volunteers. They were the maximum velocities of anterior hyoid (Cohen’s $d = 2.7$) and superior laryngeal motion (Cohen’s $d = 1.8$), the extent to which laryngeal elevation peak velocity exceeded that of hyoid elevation (Cohen’s $d = 1.6$), (Fig. 4), and the degree of movement smoothness during laryngeal elevation (Cohen’s $d = 1.8$) (Fig. 8). Among these measures, those that quantified abnormal laryngeal elevation also correlated significantly with penetration/aspiration severity. Lower laryngeal elevation peak velocity by itself, as well as relative to hyoid elevation peak velocity, were associated with more severe penetration/aspiration. On the other hand, hyoid elevation peak velocity did not differ between healthy volunteers and patients (Fig. 4). These findings suggest that greater speed of hyoid elevation by itself is not necessary for laryngeal vestibule closure to protect the airway from penetration. Instead, vestibule closure may be compromised if the maximum speed of laryngeal elevation is unable to override the maximum speed of hyoid elevation for closing the vestibule between the hyoid and larynx. Among the swallows of healthy volunteers, the occurrence of maximum vestibule closure velocity between the hyoid and larynx was strongly related to the occurrence of laryngeal elevation peak velocity, but not to the occurrence of hyoid elevation peak velocity (Fig. 6). On the other hand, time of peak negative vestibule closure velocity in patient swallows was unrelated to the times of hyolaryngeal peak velocities (Fig. 6). In other words, normal swallowing might be characterized by temporal coordination between laryngeal movement and vestibule closure, but this was not evident in the swallows of patients. These findings suggest that timely vestibule closure to protect the airway from penetration may require
early initiation of rapid upward laryngeal motion after swallow related movement has begun, as well as higher laryngeal elevation velocity to overcome hyoid elevation velocity.

Patients were delayed in achieving peak laryngeal elevation velocity during swallowing (Fig. 5), but not so in achieving peak velocity in vestibule closure compared to healthy volunteers. One possible reason is that instead of timely, rapid and smooth laryngeal elevation, some patients might have produced abnormal early depression of the hyoid, or rapid but transient laryngeal elevation. The higher number of recursive back and forth or up and down movements of the hyoid and larynx in patient swallows (Fig. 8) suggest that unsmooth movement spurs might contribute to larynx to hyoid approximation early on during the swallow. However, these types of unsmooth and extraneous movements may not effectively protect the airway from bolus penetration if rapid approximation between the larynx and hyoid is only transient and not sustained by smooth and continuous laryngeal elevation motion. This is supported by the relationship between greater penetration/aspiration severity and a lesser degree of smoothness in laryngeal elevation.

In this study, we showed that the larynx had to move smoothly, quickly enough and achieve rapid movement early enough during swallowing to achieve its movement target of vestibule closure, and similarly for anterior hyoid motion. Swallowing may be regarded as a type of ballistic action, as it involves high neural and muscular firing rates within a short duration, as well as sequential disinhibition, activation and inhibition of oral, pharyngeal and esophageal muscle activities during a swallow cycle (11). Ballistic movements are characterized by their velocity and acceleration profiles rather than movement onsets, as the focus is on producing rapid movements to reach movement targets in a short time (1). Quick and smooth movement of the larynx for airway protection is particularly important when swallowing thin liquid. Due to more rapid bolus flow through the pharynx than solids or thicker fluids, swallowing thin liquid safely may require earlier rapid laryngeal elevation and greater laryngeal movement velocity to close the vestibule quickly. The mechanism of epiglottic inversion proposed by VanDaele et al. (47)
and Logemann et al. (22) explains how anterior hyoid and superior laryngeal motions may interact to effect laryngeal vestibule closure during swallowing. Based on this model, hyoid elevation may initiate laryngeal elevation through upward traction, but thyrohyoid muscle contraction may be more crucial for elevating the larynx towards the hyoid, bringing the epiglottis to a more horizontal position (22, 47). Anterior hyoid movement may be important for 2 reasons. First, this directs the hyoid away from the path of the elevating larynx so that the larynx continues to obliterate the supraglottic airway below the epiglottis (47). Secondly, anterior hyoid movement may stretch the lateral hyoepiglottic ligaments between epiglottis and the hyoid, which bend the tip of the epiglottis to cover the larynx with assistance from base of tongue retraction (22, 47). Because these events are thought to be biomechanically driven (22, 47), the velocity, smoothness and coordination of laryngeal elevation and anterior hyoid movement may be crucial in synchronizing these events. Several observations in the swallows of healthy volunteers support this notion. Firstly, maximum laryngeal elevation and anterior hyoid movement velocities were the highest among the hyolaryngeal peak velocities, and laryngeal elevation peak velocity always exceeded hyoid elevation peak velocity (Fig. 4, healthy volunteers). These likely facilitated larynx to hyoid approximation for vestibule closure. In normal swallows, the velocity vs. time tracings for laryngeal elevation had fewer zero crossings than other hyolaryngeal movements (Fig. 8). This suggests less room for movement error in laryngeal elevation and the need for smooth motion in closing the vestibule. Earlier occurrence of maximum speed of reduction in vestibule area correlated strongly with earlier rapid laryngeal elevation and rapid anterior hyolaryngeal movement, but not with hyoid elevation (Fig. 6). This differs slightly from past research reporting a strong correlation between peak hyoid velocity in the anterior-superior direction and the occurrence of vestibule closure in normal swallowing (34). It is possible that anterior hyoid movement velocity may contribute more to this relationship than superior hyoid velocity. The role of hyoid elevation in vestibule closure may be secondary to that of laryngeal motion and anterior hyoid movement. Hyoid elevation during swallowing may
initiate laryngeal elevation, but thyrohyoid muscle contraction may be the main driver in accelerating laryngeal elevation for vestibule closure (47). This may explain why patients and healthy volunteers had similar magnitudes of hyoid elevation peak velocity (Fig. 4).

Our results are consistent with the proposed relationships between hyolaryngeal kinematics, vestibule closure and penetration/aspiration status in clinical studies. Patients with Parkinson’s disease, most of whom had penetration/aspiration during swallowing, had more velocity peaks exceeding 20 mm/s in anterior-superior hyoid motion compared to healthy individuals (19). We found the same difference between patients and healthy volunteers using the number of zero crossings in the velocity time series of hyolaryngeal movements. This measure was more suitable for quantifying movement smoothness in our data, as a large proportion of the hyolaryngeal movements of patients had peak velocities below 20 mm/s (Fig. 4, patients). Our results also agree with those of Kahrilas et al. (13), who found that delayed and reduced peak movement velocities in laryngeal elevation and anterior hyoid movement were associated with more severe penetration. They proposed that penetration was due to delayed timing and reduced speed contributed to delayed laryngeal vestibule closure and delayed UES opening (13).

However, we did not find differences between patient swallows with and without UES opening in measures of hyolaryngeal movement velocity, timing, or patterning. In a separate study, we found that measures of hyolaryngeal displacements were also similar between patient swallows with and without UES opening, even after normalizing for anatomical differences among individuals (Wong et al. b, in preparation). A possible explanation is that some patients might have abnormalities in UES relaxation, which may be contributed by impaired brainstem disinhibition of tonic contraction of the cricopharyngeus muscle (52) rather than abnormal hyolaryngeal movements.

A limitation in this study has been discussed previously (Wong et al. b, in preparation), in that the dichotomous classification of UES opening status as 2 discrete groups of swallows might have obscured different degrees of bolus clearance through the sphincter. The extent of
UES opening during swallowing as a continuous variable might be better related to measures of hyolaryngeal displacement. The healthy volunteers were on average younger than the patients. Although older individuals may initiate swallowing later than younger individuals, when the bolus has traveled deeper into the pharynx (32), our measure of time to peak movement velocity was not time-referenced to bolus position. The effect of age on maximum hyolaryngeal velocity magnitudes is unknown, therefore the increased age of the patients may or may not have contributed to differences between patients and healthy volunteers. The large effect sizes in differences between patients and healthy volunteers in this study are unlikely to be explained by age effects alone. The patients in this study were concurrently participating in other dysphagia treatment research protocols, which tend to attract more severely impaired patients who did not benefit from conventional therapy. As noted previously (Wong et al. b, in preparation), patients with milder forms of dysphagia might have been under-represented in this sample, and the group differences in this study might be greater than in typical patient populations with a more distributed range of dysphagia severities.

The results of this study demonstrated that the larynx has to initiate rapid movement early enough and achieve maximum movement velocity that can override the velocity of the elevating hyoid to close the laryngeal vestibule. In contrast, it may not be necessary for hyoid elevation to achieve faster movement speed. This may even be contraindicated in patients with slow and unsmooth laryngeal elevation movements for laryngeal vestibule closure, as moving the hyoid farther away from the larynx may open the vestibule even more and increase penetration risk. It is important to distinguish between hyolaryngeal movements that approximate normal swallowing and those that are counter-productive to safe swallowing, so that appropriate targets can be set for intervention. This will also help to identify unhelpful movement compensations that may result from the patient’s own maladaptation to the swallowing impairment, or mis-directed swallowing therapy intervention (21). Compensatory strategies and swallowing rehabilitation effort could be directed toward increasing laryngeal elevation speed, movement
smoothness and the promptness of rapid anterior hyoid and superior laryngeal movements for airway protection. Appropriate outcome measures for laryngeal elevation movement may be the extent to which laryngeal elevation peak velocity magnitude exceeds hyoid elevation velocity, the time at which peak velocity occurs within the swallow cycle, and the number of zero crossings in velocity over time. Although the magnitude and coordination of other events in the pharyngeal swallow, such as base of tongue retraction, pharyngeal contraction and pharyngeal shortening are beyond the scope of this study, identifying in future research the spatial and temporal aspects of these events that are crucial for swallowing safety and efficiency, and those that are detrimental, will complement existing knowledge on hyolaryngeal kinematics in swallowing. These new knowledge will contribute to the comprehensive rehabilitation of swallowing impairment.

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GRANTS

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REFERENCES


FIGURE LEGENDS

Figure 1.
Left lateral view of a fluoroscopic video frame. The y-axis intersects the anterior-inferior corners of the 2nd (C2) and 4th (C4) cervical vertebrae. The x-axis is at 90° to y and intersects the anterior-inferior corner of C4. Positions of the hyoid (anterior-inferior corner of the hyoid bone), larynx (anterior-superior corner of the subglottic air column), and the posterior-superior corner of the subglottic air column are tracked in the x and y dimensions during swallowing. The hyoid bone and the superior aspect of the subglottic air column are outlined. Segmental distances between the hyoid, larynx and the posterior-superior corner of the subglottic air column form a triangle indicative of the area of the vestibule between the hyoid and the larynx.

Figure 2.
Top: Superior laryngeal (LY) displacement time series of a healthy participant’s swallow. Swallow offset is the time when the larynx reached its lowest y position after peak elevation (dotted line on the right).
Middle: Velocity time series of anterior hyoid (HXvel) and anterior laryngeal (LXvel) movements in the same swallow. Positive velocity is associated with forward motion, negative velocity with backward motion. Points a and b are the first zero crossings (in either positive or negative direction) in HXvel and LXvel respectively, that led to velocity magnitude greater than +10 % or -10 % of the maximum anterior hyoid velocity (HXvelmax, Point e) and anterior larynx velocity (LXvelmax, Point f) respectively.

Bottom: Velocity time series of superior hyoid (HYvel) and superior laryngeal (LYvel) movements in the same swallow. Positive velocity is associated with upward motion, negative velocity with downward motion. Points c and d are occurrences of the first zero crossing of the
hyoid or larynx in either positive or negative direction, that led to velocity magnitude greater than
+10 % or -10 % of the maximum superior hyoid velocity (HYvelmax, Point g) and superior
laryngeal velocity (LYvelmax, Point h) respectively. Among points a to d, point c was the
swallow onset (dotted line on the left), as it was the earliest of the 4 zero crossings.

Figure 3.
Velocity time series of laryngeal elevation movement (LYvelocity) of a healthy volunteer (top)
and patient (bottom), showing the identification of zero crossings.

Figure 4.
Comparisons between healthy volunteers (left boxplot) and patients (right boxplot) on measures
of maximum velocity magnitudes. Top, from left: HXvelmax, HYvelmax, LXvelmax and
LYvelmax, in mm/s. Bottom: LYHYvelDiff, in mm/s.

Figure 5.
Comparisons between healthy volunteers (upper boxplot) and patients (lower boxplot) on the
occurrence of maximum hyolaryngeal movement velocities relative to swallow onset, from top:
HXvel_ms, HYvel_ms, LXvel_ms, LYvel_ms.

Figure 6.
Relationships between the occurrence of most rapid reduction in vestibule area
(HLarea_rapid_ms) and the occurrences of peak hyolaryngeal movement velocities (HXvel_ms,
HYvel_ms, LXvel_ms, LYvel_ms) across the swallows of healthy volunteers (filled circles) and
patients (crosses). Solid lines are the linear trend lines associated with healthy volunteers, dotted
lines are the trend lines for patients.
Figure 7.

*Top:* Time series from swallow onset (0 ms or 0 % of swallow duration) to swallow offset of a healthy volunteer, showing change in laryngeal vestibule area and hyolaryngeal displacements (anterior hyoid, HX; superior hyoid, HY; anterior larynx, LX; superior larynx, LY).

*Bottom:* Time series showing the rate of change in laryngeal vestibule area (Vestvel) and anterior hyoid (HYvel) and superior laryngeal (LYvel) velocities over the same duration as above, in the same individual.

Figure 8.

Comparisons between healthy volunteers (left boxplot) and patients (right boxplot) in the number of zero crossings in the velocity time series of hyolaryngeal movements (HXzerocross, HYzerocross, LXzerocross, LYzerocross).

Figure 9.

Comparisons between healthy volunteers (left boxplot) and patients (right boxplot) in the occurrence of maximum hyolaryngeal movement velocities relative to the total swallow duration: HXvel_%, HYvel_%, LXvel_%, LYvel_%. 
FIGURES

Figure 1.
Figure 3.

Healthy volunteer

![Graph showing LY velocity (mm/s) for a healthy volunteer with time (s) on the x-axis and LY velocity (mm/s) on the y-axis, highlighting zero crossings.]

Patient

![Graph showing LY velocity (mm/s) for a patient with time (s) on the x-axis and LY velocity (mm/s) on the y-axis, highlighting zero crossings.]

Figure 4.

Significant difference, \( p < .001 \)

NS, \( p = .007 \)
Figure 5

Time from swallow onset (ms)

GROUP
- Swallows of healthy vol
- Swallows of patients
Figure 6.
Figure 7.
Figure 8.

![Box plots showing the number of zero crossings for different trials.](image)

NS, p = .006
Figure 9.

Percentage of swallow duration

- **HXvel_%**: p < .001
- **HYvel_%**: p = .001
- **LXvel_%**: p < .001
- **LYvel_%**: NS, p = .02

Healthy volunteers | Patients
Healthy volunteers | Patients
Healthy volunteers | Patients
Healthy volunteers | Patients
Conclusion

Three studies were conducted to examine hyoid and laryngeal kinematics and swallow patterning in healthy controls and patients with dysphagia. The first study determined anatomical factors that predicted hyolaryngeal displacement magnitudes in normal swallowing. Variability in the extent of laryngeal elevation during swallowing was explained by differences in the degree of laryngeal vestibule opening at rest. The extent to which laryngeal elevation exceeded hyoid elevation during swallowing predicted the amount of closure in the laryngeal vestibule between the hyoid and larynx. Hyoid and laryngeal positions at rest before swallow onset also predicted the extent of hyoid elevation and anterior laryngeal displacement. As larynx to hyoid approximation for vestibule closure and forward hyolaryngeal displacement away from the cervical spine for UES opening are two important movement goals for swallowing, these findings suggest that hyolaryngeal movements for swallowing may be scaled by the amount of movement required to achieve these movement targets for safe swallowing. This might explain why normalizing maximum hyolaryngeal displacements by individual differences in anatomy and requirements for safe swallowing substantially reduced variability between individuals.

The second study determined the extent to which patients with dysphagia were impaired in achieving the hyolaryngeal movement targets required for safe swallowing. Raw and normalized measures of hyolaryngeal displacements and the extent of vestibule closure were compared for their ability to differentiate between the swallows of patients and healthy individuals. Measures normalized by anatomical requirements for safe swallowing contrasted between normal and disordered swallows better, especially in cases where un-scaled measures from a patient and a healthy individual would have been similar. This confirmed the notion of goal-directed movement scaling reported in other skilled motor control functions such as speech (Riely & Smith, 2003). Disordered function may be characterized by insufficient movement
relative to the requirements of the task, and not just reduced movement alone. In swallowing, insufficient laryngeal elevation to overcome the extent of hyoid elevation might be detrimental to laryngeal vestibule closure and airway protection in patients with dysphagia. This suggests that instead of measuring hyoid elevation magnitude as an outcome for quantifying movement impairment in swallowing, measuring the difference between maximal laryngeal elevation and hyoid elevation magnitudes may better capture the degree to which the laryngeal vestibule can be closed and protected from penetration during swallowing. In swallowing rehabilitation, improving hyoid elevation alone may not improve swallow function and safety; sufficient laryngeal elevation is needed for vestibule closure. The extent of anterior hyoid displacement did not appear to be scaled by differences in anatomy, and its raw measure differentiated between normal and disordered swallowing.

In the third study, measures of hyolaryngeal movement timing, velocity and patterning were compared between the swallows of patients and healthy individuals. In many aspects, results in the second and third studies were consistent. Similar to displacement magnitudes, anterior hyoid and superior laryngeal movement velocity, timing and patterning were also more impaired in patients compared to other measures of anterior laryngeal and superior hyoid movement. In contrast, the maximum velocity of hyoid elevation did not differ between the swallows of healthy individuals and patients with dysphagia. In healthy individuals, the occurrence of laryngeal vestibule closure peak velocity was related to the occurrence of laryngeal elevation peak velocity, but not to the occurrence of hyoid elevation peak velocity. Laryngeal vestibule closure may therefore relate more closely with laryngeal elevation than hyoid elevation based on maximal displacement measures and temporal measures. On the other hand, patient swallows did not exhibit relationships between time of peak vestibule closure velocity and time of hyolaryngeal peak velocities. This may be indicative of impaired movement coordination for vestibule closure in disordered swallowing. Reduced smoothness and rapidity of laryngeal elevation also correlated with penetration/aspiration severity. Collating the findings from the
second and third studies, the larynx may need to displace sufficiently, smoothly, quickly enough and achieve rapid movement early enough during swallowing to overcome upward movement of the hyoid to achieve the goal of vestibule closure.

Spatial and temporal kinematic measures are complementary in quantifying movement control and coordination, and may be more sensitive in detecting differences in impairment than qualitative clinical measures (Krasovsky & Levin, 2010; Levin, Kleim, & Wolf, 2009; Rohrer et al., 2002). The 3 studies have derived objective measures of hyolaryngeal displacements, movement timing and patterning. These measures may provide sufficient distinction between normal and disordered swallowing function to be applied to a clinical population for quantifying movement impairment in swallowing. They should be tested in future research for their ability to detect changes associated with dysphagia intervention or recovery.
References


