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SCHOOL OF MEDICINE

Dissertation

A TWO-SLING MECHANISM OF HYOLARYNGEAL ELEVATION IN THE PHARYNGEAL PHASE OF SWALLOWING

by

WILLIAM GORDON PEARSON, JR.

B.S., University of Georgia 1986

Submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

2012

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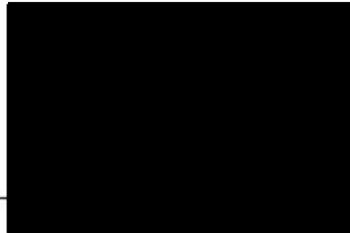

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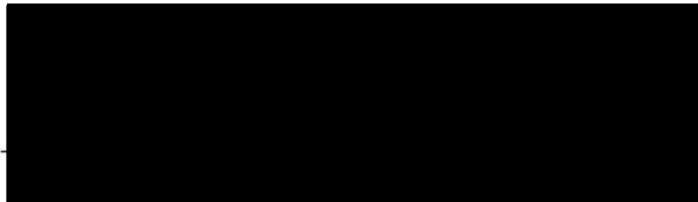

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DEDICATION

In memoriam,

Col. William G. Pearson, MSC, US Army Retired

A man of faith, science, and service

1926-2012

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There are two seemingly unrelated obligations in acknowledgement. The first is to accept the truth, and the second is to express gratitude. Common to both obligations is the discipline of recognition: the more I recognize my limitations, the more I recognize the contributions of others. What I did not recognize at the beginning of this journey is how much I would be dependent upon others to complete it. I had the false notion that to earn a doctor of philosophy would purely be a personal achievement. Perhaps there are those individuals who are able to attain this kind of personal achievement on their own; in my case, this personal attainment was received. I received wisdom, guidance, and encouragement from investigators who have carefully documented their findings, from those around me who have taken an interest in this project, and finally from God, who inspires my daily work. If a doctor of philosophy is one who "teaches doctrine of the love of wisdom", then my training has taught me that wisdom is received, not earned. Dr. Peter Bergethon named this for me when he said, "All great scientists know that knowledge comes from outside them. It is grace." I do not aspire to be a great scientist, but I am the happy recipient of grace, to which gratitude is the appropriate response. How else does one receive a gift that surpasses merit? In these lines I would like to offer my gratitude to those who have been the embodiment of grace to me.

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Breaking bread with others was important to Jesus. It is an essential part of human flourishing. My hope is that our collaboration will become a gift to those who struggle to flourish.

A TWO-SLING MECHANISM OF HYOLARYNGEAL ELEVATION IN THE PHARYNGEAL PHASE OF SWALLOWING

(Order No.)

WILLIAM GORDON PEARSON, JR

Boston University School of Medicine, 2012

Major Professor: Ann C. Zumwalt, Ph.D., Asst. Professor of Anatomy and Neurobiology

ABSTRACT

The pharyngeal phase of swallowing is a complex function that transfers a bolus from the oral cavity through the hypopharynx into the esophagus. A critical event in this process is the elevation of the hyolaryngeal complex, which opens the upper esophageal sphincter and relocates the airway away from an oncoming bolus. The suprahyoid group of muscles (mylohyoid, geniohyoid, digastric, and stylohyoid) and thyrohyoid are thought to underlie this function. The role of a deeper posterior sling of muscles that is comprised of stylopharyngeus, salpingopharyngeus and palatopharyngeus has not been determined. This project aims to investigate a hypothesized two-sling mechanism for hyolaryngeal elevation in the pharyngeal phase of swallowing.

The thesis begins with background information of the functional anatomy thought to underlie hyolaryngeal elevation followed by an outline of studies that validate the structure, function, and clinical relevance of the two-sling mechanism. A cadaver model is first used to calculate potential force vectors of the muscular slings. The function of the two-sling apparatus is then investigated *in vivo* by using muscle functional MRI to evaluate muscles active in swallowing and dynamic MRI to perform kinematic analysis on key anatomical landmarks that represent attachment sites of the two-sling mechanism. Finally, the clinical significance of the two-sling mechanism is demonstrated by comparing spatial and temporal measurements collected from fluoroscopic imaging studies of patients with normal swallowing ability and swallowing difficulty.

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ABBREVIATIONS

ADG	Anterior digastric
AMS	Anterior muscular sling
ANOVA	Analysis of variance
C2,C4, etc.	Cervical vertebrae and corresponding number
CN	Cranial Nerve
CPG	Central pattern generator
CT	Computed Tomography
CV	Canonical variate
CVA	Canonical variate analysis
DICOM	Digital imaging and communication in medicine
EPG	Effortful pitch glide
FEES	Fiberoptic Endoscopic Examination of Swallowing
GH	Geniohyoid (GH)
HNC	Head and neck cancer
HSD	Honestly Significant Difference
HyEx (mand)	Hyoid excursion measured against the axis of the mandible
HyEx (vert)	Hyoid excursion measured against the axis of the vertebrae
HyLx Approx	Hyolaryngeal approximation
IMRT	Intensity- modulated radiotherapy
LxEl	Posterior laryngeal elevation
MBS	Modified barium swallows (MBS)
mfMRI	Muscle functional magnetic resonance imaging
MH	Mylohyoid
MM	Mendelsohn maneuver
MRI	Magnetic resonance imaging
NRRS	Normalized Residue Ratio Scale

PAS	Penetration-Aspiration Scale
PC	Principal component
PCA	Principal components analysis
PCR	Pharyngeal Constriction Ratio
PCSA	Physiological cross sectional area
PDG	Posterior digastric
Phx short	Pharyngeal shortening
PMS	Posterior muscular sling
RV	Random variable
SD	Standard Deviation
SH	Stylohyoid
T1	Longitudinal relaxation time
T2	Transverse relaxation time
TE	Echo time
TR	Repetition time
UES	Upper esophageal sphincter
WS	Wallenberg's syndrome

Chapter 1

Introduction: A Two-Sling Mechanism of Hyolaryngeal Elevation in the Pharyngeal Phase of Swallowing

Abstract

This chapter sets the context and outlines the investigative flow of the thesis entitled: A Two-Sling Mechanism of Hyolaryngeal Elevation in the Pharyngeal Phase of Swallowing. The chapter begins with a rationale for investigation of the functional anatomy thought to underlie hyolaryngeal elevation. While it has long been known that the suprahyoid muscles (geniohyoid, mylohyoid, digastric, and stylohyoid) and the long pharyngeal muscles (palatopharyngeus, salpingopharyngeus, and stylopharyngeus) suspend the hyolaryngeal complex (composed of the hyoid, larynx, and elements of the pharynx), the contribution of these muscles to pharyngeal swallowing is not fully established. Background information from the literature reveals gaps of knowledge and inconsistencies concerning the anatomy underlying hyolaryngeal elevation. Subsequent sections provide an overview of the present study and set forth a set of systemic inquiries to investigate the structure, function, and clinical relevance of the two-sling mechanism of hyolaryngeal elevation.

Chapter 1

Introduction: A Two-Sling Mechanism of Hyolaryngeal Elevation in the Pharyngeal Phase of Swallowing

The pharyngeal phase of swallowing is a complex cascade of events that utilizes over twenty muscles and multiple skeletal elements to transform a respiratory channel into a digestive conduit and effectively transfer a bolus from the oral cavity through the hypopharynx into the esophagus. The critical event in the pharyngeal phase of swallowing is the elevation of the hyolaryngeal complex that relocates the larynx anteriorly away from the trajectory of an oncoming bolus and stretches open the upper esophageal sphincter (Matsuo and Palmer, 2008; Olsson et al., 1997). The hyolaryngeal complex is comprised of hyoid bone, larynx, and associated structures, including the cricopharyngeus muscle that forms the upper esophageal sphincter (Fig. 1.). The hyolaryngeal complex connects the pharynx with the trachea and esophagus. Reduced hyolaryngeal elevation is associated with aspiration in post-stroke and post-radiation head and neck cancer patients, underscoring the importance of this function in airway protection (Bingjie et al., 2010; Pauloski et al., 2006). Laryngeal elevation has been shown to be more important to bolus clearance than pharyngeal constriction. In one study reduced laryngeal elevation was associated with retention of the bolus in the hypopharynx, whereas pharyngeal pressures were not (Olsson et al., 1997). This dissertation investigates the functional anatomy underlying this critical event in swallowing.

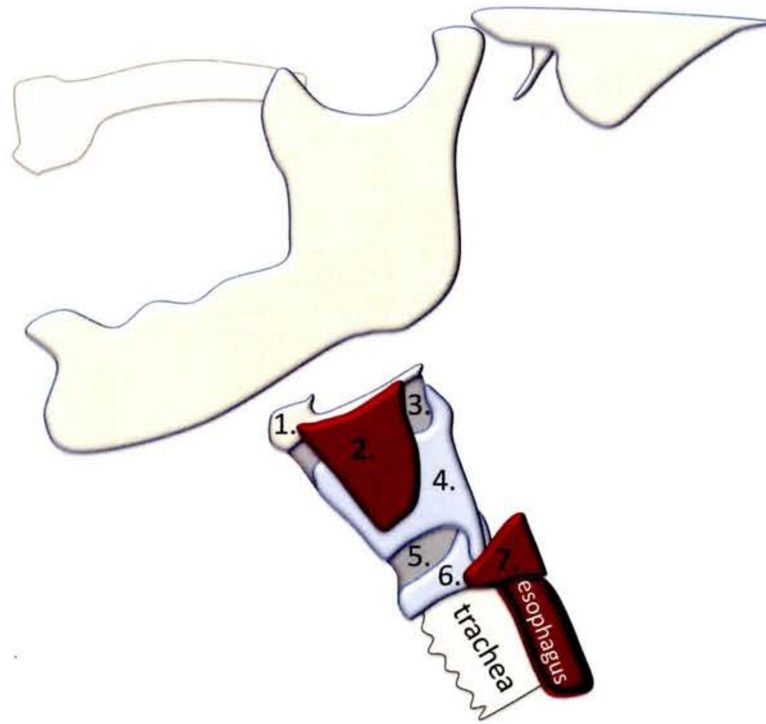

Fig. 1. The hyolaryngeal complex includes the: 1.) hyoid bone, 2.) thyrohyoid muscle 3.) thyrohyoid membrane, 4.) thyroid cartilage, 5.) cricothyroid membrane 6.) cricoid cartilage and 7.) cricopharyngeus. The tonic contraction of the cricopharyngeus is the primary source of the high-pressure zone referred to as the upper esophageal sphincter. This high-pressure zone is also referred to as the pharyngeal esophageal segment. The hyolaryngeal complex incorporates the trachea and esophagus.

Background for the Two-Sling Mechanism of Hyolaryngeal Elevation

Throughout the literature, various muscles have been implicated as relevant to hyolaryngeal elevation including: geniohyoid, mylohyoid, digastric, stylohyoid, thyrohyoid, palatopharyngeus, salpingopharyngeus, and stylopharyngeus (Fig. 2). Each of these muscles and its presumed function has been described (Lockhart et al., 1969). In humans, these muscles have been validated as active during swallowing with intramuscular electromyography except for stylopharyngeus, as this muscle cannot be

accessed with electrodes (Kurt et al., 2006; Palmer et al., 2008; Van Daele et al., 2005). In spite of the importance of this critical event in deglutition, the description of the functional anatomy underlying hyolaryngeal elevation in swallowing has gaps and is sometimes inconsistent in the literature.

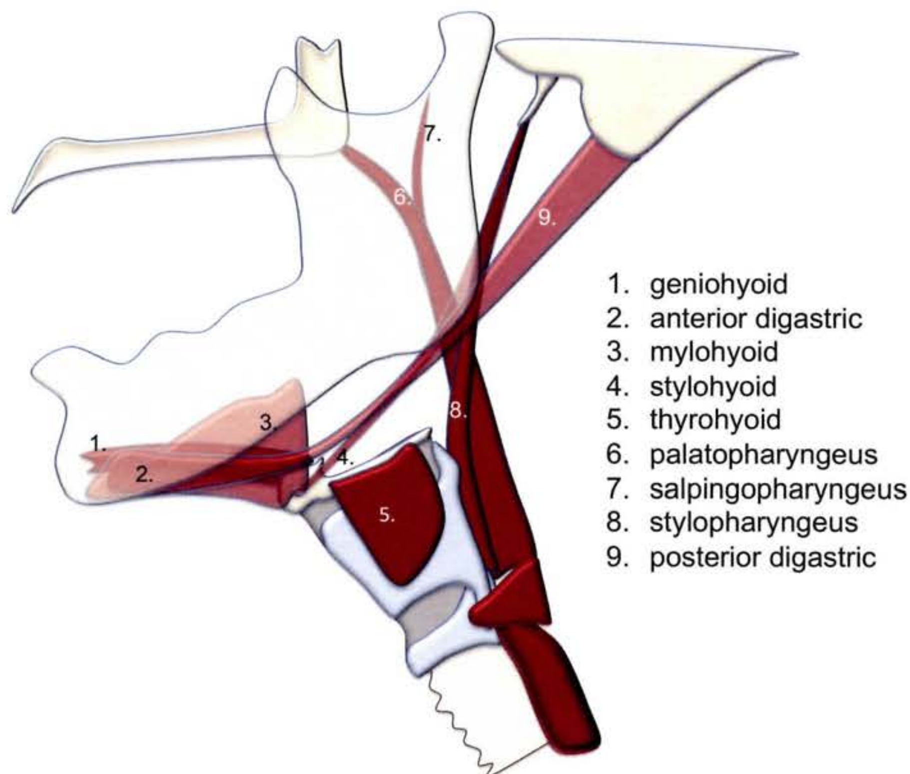

Fig. 2. Muscles suspending the hyolaryngeal complex

The most common description is that some combination of the suprahyoid muscles (geniohyoid, mylohyoid, stylohyoid, anterior digastric, and posterior digastric), or the submental muscles (geniohyoid, mylohyoid, anterior digastric), aided by the thyrohyoid muscle elevates the hyolaryngeal complex and therefore opens the upper esophageal sphincter (Cook et al., 1989; Ertekin and Aydogdu, 2003; Matsuo and Palmer, 2008;

Miller, 1982). The electrical activity of the geniohyoid, mylohyoid, and thyrohyoid muscles during swallowing has been documented in animals with electromyography (Thexton et al., 2007). There are data associating laryngeal elevation with submental muscle activity (Ertekin et al., 2001). The anterior movement of the hyoid aided by the action of the thyrohyoid muscle to approximate the larynx to the hyoid is described in the swallowing literature as responsible for hyolaryngeal elevation (Cook et al., 1989).

In human subjects research, hyoid elevation is a primary focus for improving swallowing outcomes. The Shaker swallowing exercise regimen is thought to target the suprahyoid and thyrohyoid muscles (Mepani et al., 2009). A compensatory technique called effortful swallow was shown to increase hyoid elevation (Hind et al., 2001). Experimentation with intramuscular electrical stimulation of the geniohyoid, mylohyoid and thyrohyoid resulted in elevation of the hyoid and larynx (Burnett et al., 2003). Stimulation of the submental and thyrohyoid muscles has been attempted with surface electrodes (Humbert et al., 2006). Surface stimulation of the submental muscles coupled with exercise has produced transient improvement to swallowing function (Park et al., 2009). Common to each of these studies is the belief that the submental muscles and the thyrohyoid are the essential target muscles underlying hyoid movement resulting in a protected airway and stretching open a relaxed upper esophageal sphincter.

Others have proposed that the longitudinal pharyngeal muscles (stylopharyngeus, palatopharyngeus, and salpingopharyngeus) also assist to elevate the larynx and protect the airway during swallowing (Kahrilas, 1993). An electromyography study of human swallowing provided evidence that the palatopharyngeus is active (Van Daele et al., 2005). Anatomical descriptions of the stylopharyngeus and palatopharyngeus report that the distal insertions include the thyroid cartilage, indicating that these muscles have the potential to elevate the hyolaryngeal complex (Meng et al., 2008; Okuda et al., 2008).

Other clues scattered throughout the clinical literature implicate the importance of the long pharyngeal muscles in swallowing. Intensity-modulated radiation therapy is a procedure allowing clinicians to spare tissues outside of the targeted treatment area in order to minimize collateral damage to structures important to swallowing. Investigators in this field emphasize that the pharyngeal muscles (including the long pharyngeal muscles) are more important to swallowing than the submental muscles (Christianen et al., 2011; Eisbruch et al., 2004). While the method used to exclude the submental muscles are questionable, these studies call attention to the role of the long pharyngeal muscles. Studies of patients with lateral medullary infarctions (presumably affecting the nucleus ambiguus and innervating the long pharyngeal muscles) have severe dysphagia during the pharyngeal phase of swallowing (Aydogdu et al., 2001). Another clinical investigation testing a battery of swallowing exercises reported no improvement in hyoid elevation (suprahyoid muscles), but did find an increase in epiglottic retroflexion indicating that some other muscle group (i.e. the long pharyngeal muscles) may underlie laryngeal elevation (Carroll et al., 2008). Interestingly, in these last two clinical studies, the belief that the submental muscles alone are critical to laryngeal elevation was underscored.

All suprahyoid muscles and longitudinal pharyngeal muscles recorded with electromyography in animal and human studies have been shown to be active during the complicated process known as deglutition (Thexton et al., 2007; Van Daele et al., 2005). The connection between these muscles and the particular events of swallowing is inferred. Submental muscles are accessible to surface or hook wire electromyography whereas the deeper longitudinal pharyngeal muscles are not (namely stylopharyngeus) (Doeltgen et al., 2007). As noted above, data show an association between submental muscle activity, laryngeal elevation, and opening of the upper esophageal sphincter. The role of pharyngeal shortening has been assigned to the longitudinal pharyngeal muscles even though they directly, or indirectly, attach to the hyolaryngeal complex (Kahrilas PJ at al., 1992). Their role has not been determined experimentally. It is plausible that both

muscle groups function together to elevate the hyolaryngeal complex. An insufficient conceptualization of hyolaryngeal elevation in deglutition may explain some of the unanticipated findings in swallowing studies.

This thesis proposes and investigates a potential two-sling mechanism for hyolaryngeal elevation (Fig. 3). The two slings are comprised of bilateral muscles. The anterior sling is composed of the suprahyoid muscles and the posterior sling is composed of the long pharyngeal muscles. The suprahyoid muscles (mylohyoid, geniohyoid, stylohyoid, and digastric) have proximal attachments to the mandible anteriorly and to the cranial base posteriorly. The distal insertion points of the suprahyoid muscles exert force principally on the body of the hyoid bone and therefore function as an anterior sling. The posterior muscular sling consists principally of the stylopharyngeus and palatopharyngeus. The salpingopharyngeus also contributes to this sling; the proximal portion of the muscle is diminutive and the distal portions blend with the stylopharyngeus and palatopharyngeus. The palatopharyngeus attaches superiorly into the palatine aponeurosis and muscles of the velum of the soft palate. This superior attachment site is elevated and stabilized by the actions of the tensor veli palatini and the levator veli palatini (Okuda et al., 2008). Another key muscle in this complex is the thyrohyoid muscle. This is not a sling muscle that suspends the hyolaryngeal complex, but rather an intrinsic muscle of the hyolaryngeal complex. The body of work presented here investigates the possibility that these two slings function together as components of a system to elevate the hyolaryngeal complex in the pharyngeal phase of swallowing.

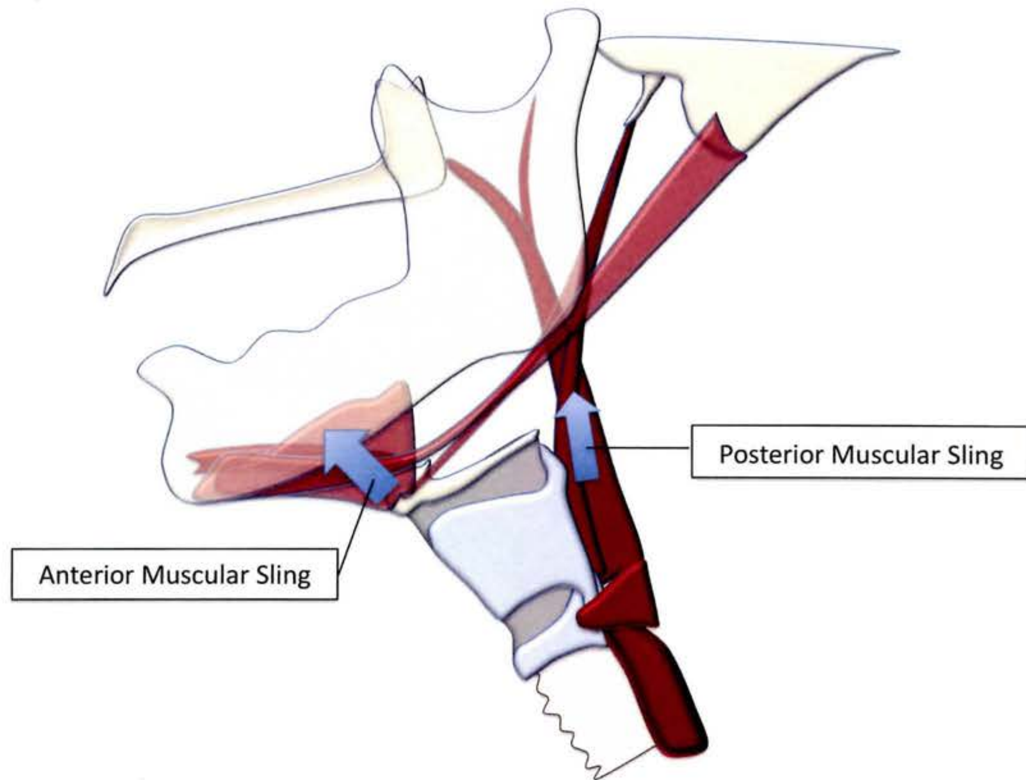

Fig. 3. Proposed two-sling mechanism for hyolaryngeal elevation is illustrated. Hyolaryngeal elevation functions to assist in protecting the airway from bolus penetration into the laryngeal aditus or aspiration thorough the vocal folds into the trachea, and to stretch open a relaxed upper esophageal sphincter.

Other events important to pharyngeal swallowing

Effective pharyngeal swallowing requires the neuromuscular coordination of several important events. These events begin with the tongue propelling the bolus into the hypopharynx. The oral cavity, nasopharynx, and larynx are subsequently closed off, converting a respiratory channel into alimentary canal (Miller, 1982) (Fig. 4). Oropharyngeal muscles elevate the hyoid and larynx, which stretches open the upper esophageal sphincter to receive the oncoming bolus (Fig. 5). The upper esophageal sphincter is a high-pressure zone at the junction of the pharynx and esophagus; it is

principally formed by the tonic contraction of the cricopharyngeus, the distal portion of the inferior pharyngeal constrictor muscle that inserts on the cricoid cartilage (Sivarao and Goyal, 2000). These events are followed a wave of pharyngeal constriction to clear the bolus from the pharynx followed by the descent of the hyoid and larynx (Kahrilas et al., 1992) (Fig. 6). This sequence of events is triggered by sensory receptors in the pharynx responding to the bolus and sending action potentials to the nuclei of the solitary tract. The solitary nuclei trigger a central pattern generator that initiates and coordinates the pharyngeal phase of swallowing (Jean, 2001) (Fig. 7).

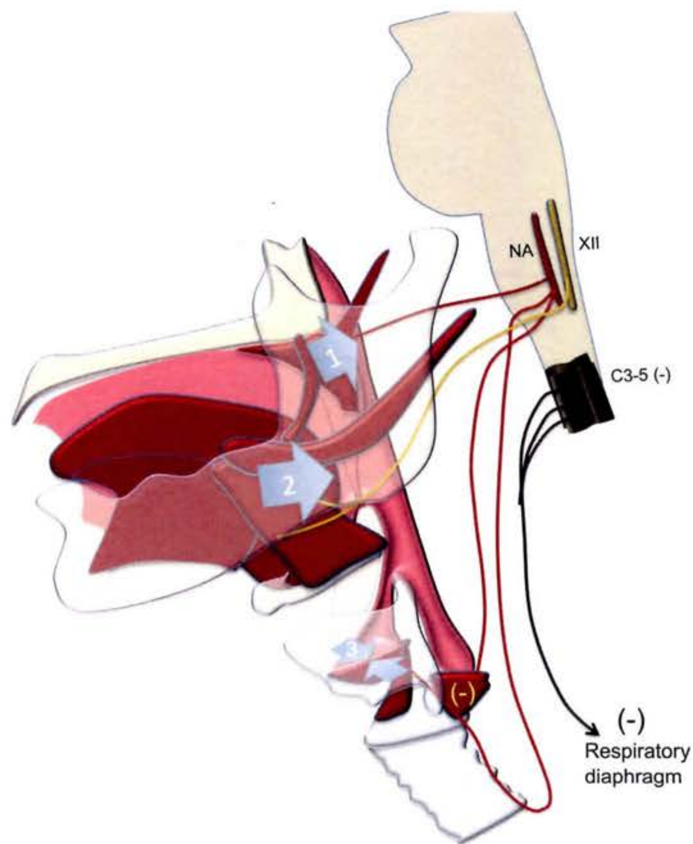

Fig. 4. Structures associated with converting a respiratory conduit into a gustatory channel are illustrated. Arrows indicate the closing off of the 1.) nasopharynx, 2.) oral cavity, and 3.) larynx. Cranial nerve nuclei associated with each valve closure are indicated. Nerve cell bodies of the vagus nerve closing the nasopharynx and larynx

reside in nucleus ambiguus (NA). Retrusion of the tongue (under the control of the hypoglossal nerve, cranial nerve XII) and the narrowing of the oropharyngeal isthmus occlude the oral cavity. The yellow (-) indicates that the tonic contraction of the cricopharyngeus is inhibited relaxing the upper esophageal sphincter. The black (-) signifies the apnea event (cessation of respiration) associated with swallowing.

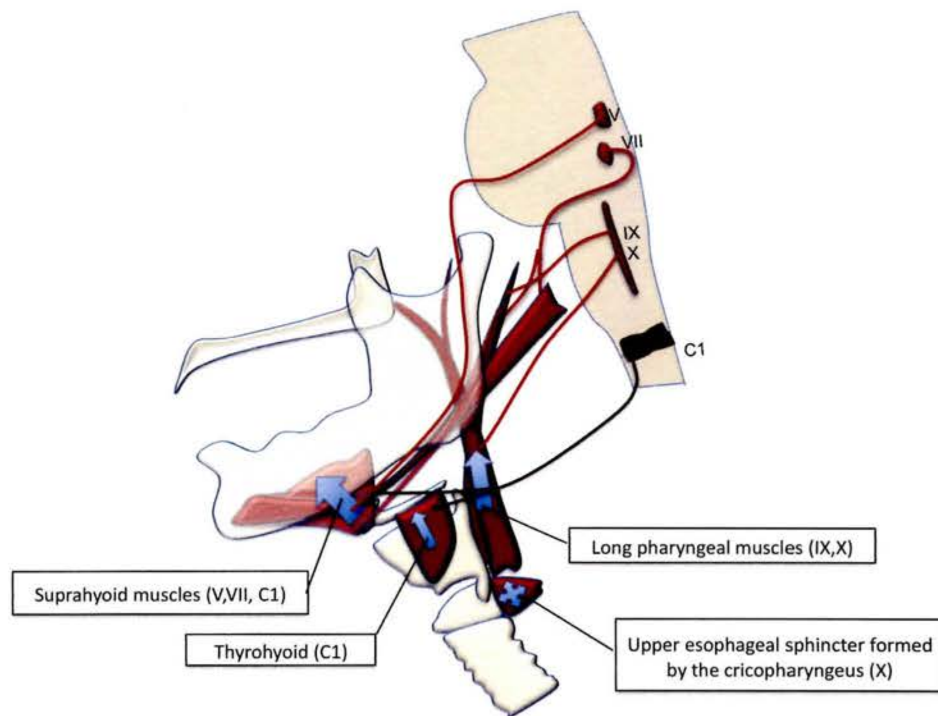

Fig. 5. Structures that potentially elevate the hyoid, larynx, and pharynx stretching open a relaxed upper esophageal sphincter. Cranial nerve involvement is noted by roman numerals. C1 = first cervical spinal nerve.

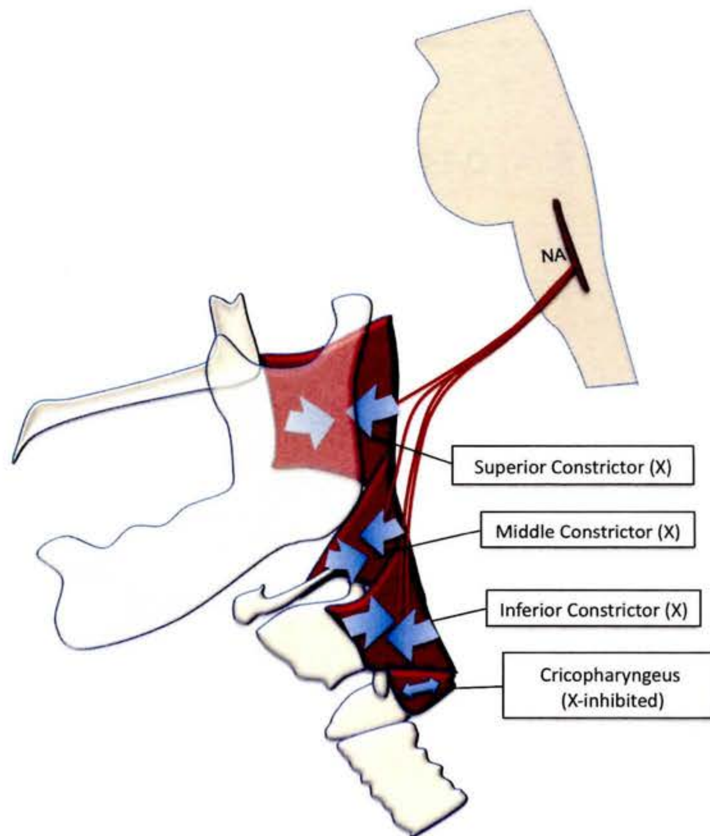

Fig. 6. Structures associated with the stripping wave of pharyngeal constriction clearing the bolus from the hypopharynx are illustrated. The pharyngeal branches of the vagus nerve (cranial nerve X) innervate the pharyngeal constrictors. The cricopharyngeus, located at the junction of the pharynx and esophagus, is inhibited during the peristaltic contraction of the pharyngeal constrictors allowing for the bolus to pass into the esophagus.

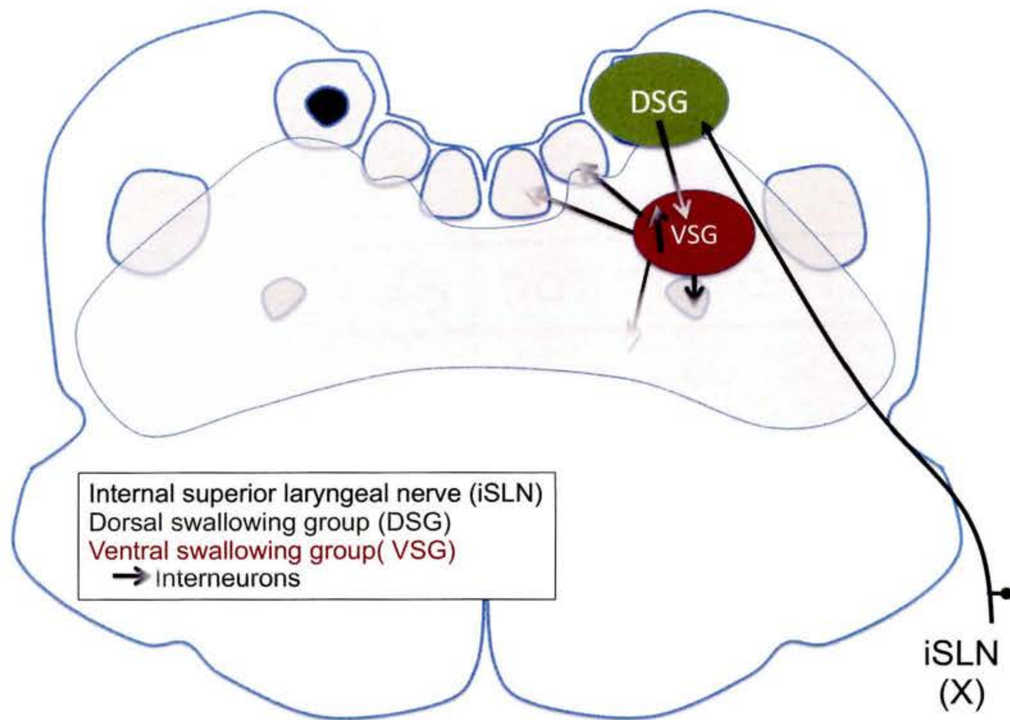

Fig. 7. Illustrated components of a brainstem swallowing central pattern generator (CPG) modulated by supramedullary input at the level of the rostral medulla (axial slice). This CPG coordinates the motor unit recruitment of muscles that are under the control of five cranial nerves (CN V, VII, IX, X, XII) and five spinal nerves (C1-5). Sensory receptors including taste, temperature, proprioception, and tactile sensation provide information that modifies the rhythmic pattern of each swallowing episode. Other regulated functions mediated by the brainstem important to pharyngeal swallowing include swallowing apnea and salivary flow.

Overview of systemic investigations in the structure underlying hyolaryngeal elevation

A series of research questions are addressed in this thesis to investigate the structure, function, and relevance of the two-sling mechanism of hyolaryngeal elevation. The first

investigations establish which muscles possess the structural potential to elevate the hyolaryngeal complex (Fig. 8, Chapters 2 and 3). The second set of question address function (Fig. 9, Chapters 4,5, and 6). In this section we ask if the muscles of the two slings are active, what actions they perform, and if they function as part of a system and therefore warrant being called a mechanism. The final set of questions relate to the clinical importance of the two-sling mechanism (Fig. 10, Chapters 7 and 8).

Structure of the Two-Sling Mechanism for Hyolaryngeal Elevation

To investigate the structure of the two-sling mechanism we used a cadaver model to study the structural properties of muscles by measuring the physiological cross sectional area (PCSA) of the muscles. PCSA provides a relative measurement of maximum tetanic tension of muscles that is used as a proxy for force potential. Using PCSA measurements and lines of muscle action calculated by three-dimensional coordinates of muscle attachments, we calculate unit force vectors of each muscle. Unit force vectors show the potential for force of a muscle in an anatomical axis of interest. In chapter 2 of this dissertation the suprahyoid muscles were evaluated to determine which of these muscles has the greatest potential to displace the hyoid anteriorly and superiorly. In chapter 3, muscle groups including the anterior muscular sling, posterior muscular sling, and the thyrohyoid were evaluated to determine which of the muscles have the greatest potential to elevate the hyolaryngeal complex. Taken together, these chapters demonstrate that an anterior muscular sling consisting of the geniohyoid, mylohyoid, stylohyoid and digastric, and the posterior sling consisting of the palatopharyngeus, salpingopharyngeus, and stylopharyngeus, along with the thyrohyoid, all have the potential to elevate the hyolaryngeal complex.

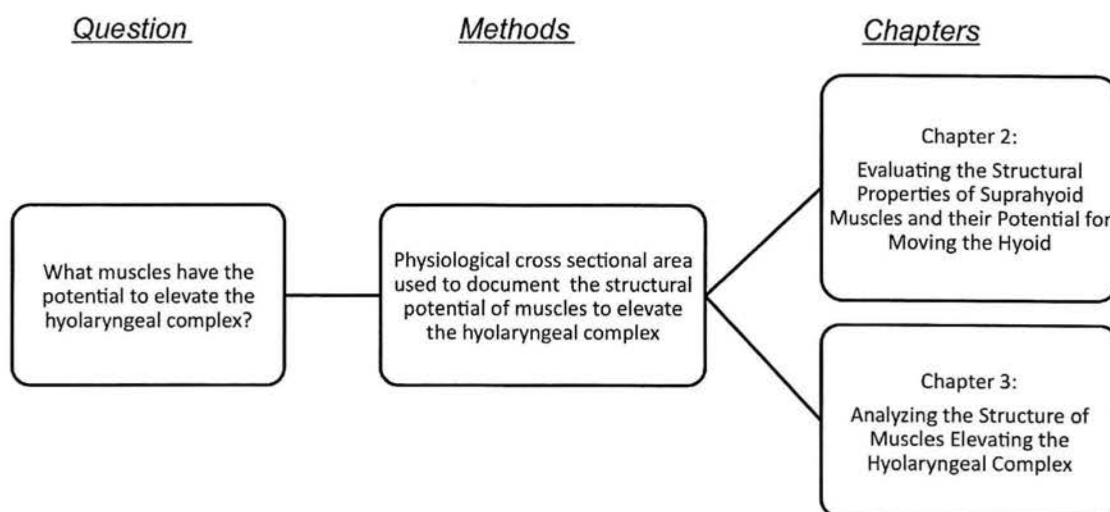

Fig. 8. Flow chart of cadaveric studies used to determine the structure of the two-sling mechanism of hyolaryngeal elevation

Function of the Two-Sling Mechanism for Hyolaryngeal Elevation

Investigations of muscle function must consider whether muscles are active during the time in question (in this case, during swallowing), and what actions these muscles perform. Intramuscular electromyography is the conventional method for determining the physiological activity of a muscle. However, this method cannot be applied to the posterior sling muscles, as they cannot be safely accessed in human subjects. An alterative for determining muscle activity is muscle functional magnetic resonance imaging (mfMRI) (Segal, 2007). Muscle activity is indicated by comparing transverse relaxation times (T2 signals) of muscles from mfMRI acquisitions before and after muscle use. The increase in T2 signal is attributable to water uptake induced by osmotic pressure changes from metabolic byproducts of muscle use.

Physiological response of a muscle does not determine the action of the muscle, it simply indicates whether the muscle was active (contracting) or not. Muscle contraction can be concentric, eccentric, or isometric. Kinematic measurements, such as the excursion of the hyoid bone or larynx, measure the movement of particular anatomic features. If the underlying anatomical structure is understood and particular muscles have been shown to be active, kinematic measurements can be used to discern the action of the underlying musculature. Kinematic measurements can be collected from dynamic MRI sequences. In this dissertation an MRI study including mfMRI and dynamic MRI acquisitions was obtained from a cohort of young healthy subjects to determine the function of swallowing muscles.

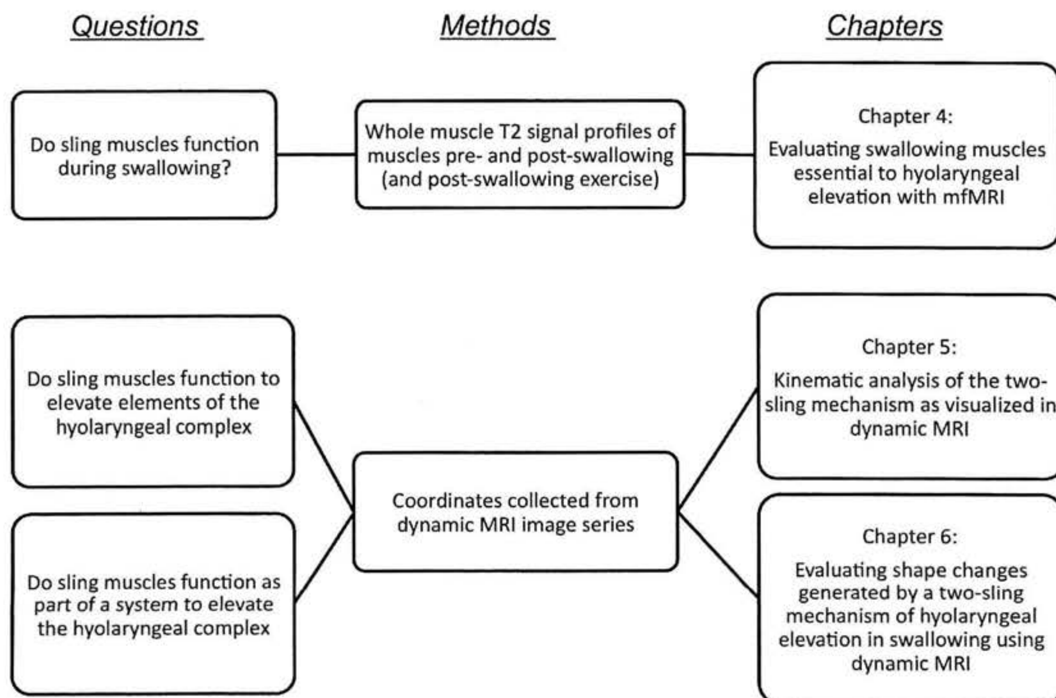

Fig. 9. Flow chart of the MRI study used to determine the function of the two-sling mechanism of hyolaryngeal elevation

In chapter 4 we document whether the muscles described in chapters 2 and 3 were active during swallowing using mfMRI. This method has been used to study the activity of muscles of mastication (Yamaguchi et al., 2011). The current study is the first to use this method to study swallowing muscles. T2 images are acquired before and after swallowing to evaluate the physiological response of presumed swallowing muscles. Transverse relaxation times of each muscle before and after swallowing were compared. In this study we found that all anterior sling muscles were active except the anterior digastric. The stylopharyngeus and palatopharyngeus of the posterior muscular sling were also found to be active. The diminutive salpingopharyngeus was excluded from this study. The thyrohyoid, reported as an important muscle in hyolaryngeal elevation in swallowing, did not register consistent activation in our cohort of subjects.

In chapter 5, we employed kinematic measurements to determine if muscles of interest elevate elements of hyolaryngeal complex. Using the insights of the anatomical studies in chapters 2 and 3, we developed a methodology using coordinates to map the movement of the two-sling mechanism in a time series of lateral view radiological images such as dynamic MRI or videofluoroscopy. In this method, coordinates of proximal attachments of the muscular slings from three skeletal levers and coordinates of distal attachment points of the muscular slings to elements of the hyolaryngeal complex are recorded at minimum and maximum hyolaryngeal excursion. Coordinates are trigonometrically transformed into distance measurements of muscle attachments at both time points. Differences in distance measurements are used to determine if muscles shorten, extend, or remain constant during hyolaryngeal excursion.

Chapter 6 documents whether the muscular and skeletal elements of the proposed mechanism function together to elevate the hyolaryngeal complex. If so, then coordinates mapping the covariant movement of the muscular slings and skeletal levers should be associated with hyolaryngeal elevation. The same set of coordinates collected

for Chapter 5 was used in Chapter 6 for morphometric analyses. Chapter 6 documents the covariance of the system using principal components analyses to determine if the two muscular slings function as the underlying mechanism of hyolaryngeal elevation. Rather than calculating distance measurements, coordinates collected from minimum and maximum hyolaryngeal excursion are used to analyze covariant shape changes. Principal components analysis is a multivariate analysis of covariance. It is used to determine the vectors of covariance for anatomical landmarks called eigenvectors. Eigenvectors are plotted to show the effect of the two-sling mechanism. These shape changes are not actual distance changes of landmarks, but rather the direction and magnitude of shape change associated with the covariance of multiple elements of a dynamic system. This study demonstrated that the covariance of coordinates representing the muscular and skeletal elements of a two-sling mechanism is associated with hyolaryngeal elevation.

Clinical Relevance of the Two-Sling Mechanism for Hyolaryngeal Elevation

Chapters 2-6 establish the structure and function of the two-sling mechanism for hyolaryngeal elevation in the pharyngeal phase of swallowing. Chapters 7 and 8 address the question of clinical relevance of the two-sling mechanism using data collected in a retrospective study of patients with dysphagia who were imaged using videofluoroscopy, a clinical imaging modality (Fig. 8).

In Chapter 7, methods developed in the previous functional studies (chapters 5 and 6) are adapted to answer the question of whether the two-sling mechanism is important to safe swallowing. Hyolaryngeal elevation is thought to be a critical function in swallowing, key to protecting the airway and stretching open the upper esophageal sphincter to receive an oncoming bolus. To test whether or not the function of the two-sling mechanism is important to favorable swallowing, swallowing kinematics of post-treatment head and neck cancer patients were compared to those of age and gender matched patients judged to have "normal" swallowing function by a speech-language pathologist.

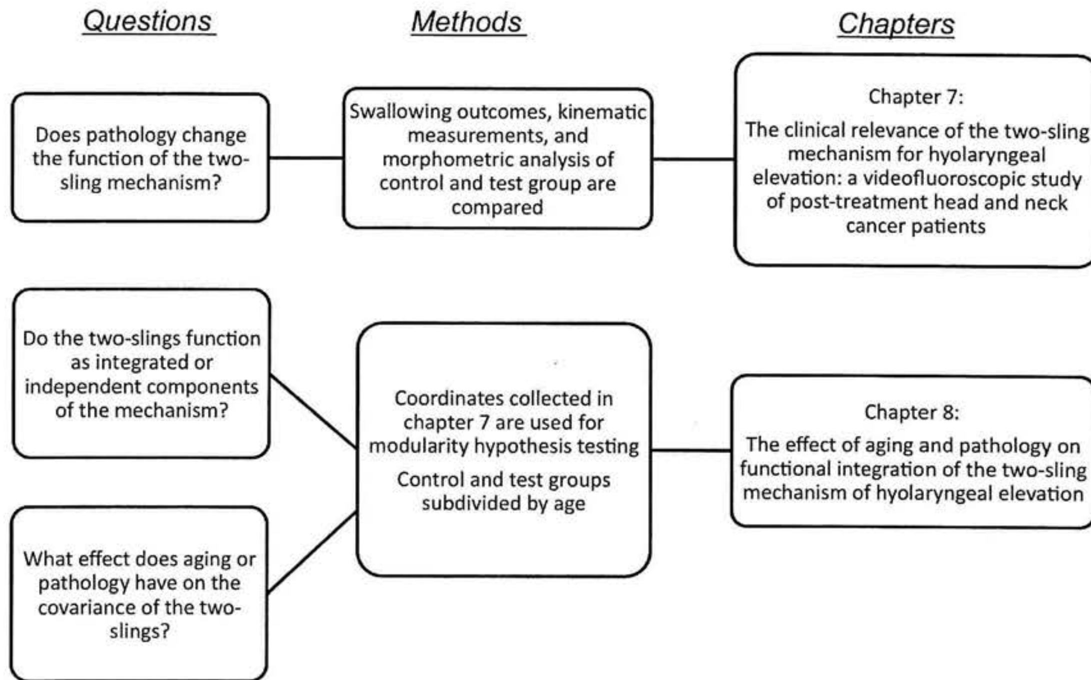

Fig. 10. Flow chart of videofluoroscopic studies used to determine the clinical relevance of the two-sling mechanism of hyolaryngeal elevation

Data were collected from patient records and videofluoroscopic swallowing studies, also called modified barium swallows. Swallowing outcomes (penetration-aspiration and residue) were assessed in both groups to verify that poor swallowing function characterized the test group as compared with the control group. Poor swallowing outcomes include: penetration of bolus into the vestibule of the larynx, aspiration of bolus through the vocal folds into the trachea, and retention of bolus in the pharynx after swallowing (residue). Kinematic measurements of the anterior and posterior slings, including the length of excursion time and the distance of excursion, were compared

between the test and control groups. Morphometric analyses evaluating shape change of the two-sling mechanism associated with hyolaryngeal elevation were compared in the two groups. The kinematic and morphological data show that the two-sling mechanism is impaired in the test group, supporting the postulate that it underlies normal swallowing physiology.

The final investigation (chapter 8) of the two-sling mechanism addresses the functional modularity of the two-slings and how this modularity is changed with age or pathology. In previous chapters, we have determined how particular muscle groups function, and that the muscular and skeletal elements of the mechanism function together as part of a system. In chapter 8 we investigate the modularity of the two slings; namely, do the elements comprising each sling function as identifiable components of the kinematic system elevating the hyolaryngeal complex. Using the same set of coordinates collected from the MBS studies in chapter 7, we use another morphological test of covariance called modularity hypothesis testing to confirm that the skeletal and muscular elements of each sling hypothesized to function as a component statistically covary with one another and thus demonstrate modularity. A second and different question addressed in this chapter is if the two slings as modules functionally covary and therefore act in an integrated or independent manner. By subdividing our test and control groups from chapter 7 by age, we also investigate the effect of age and pathology on modularity. These studies demonstrate that the skeletal and muscular elements of each sling function together as a module. Findings also suggest that the role of thyrohyoid many change with age. Finally, the two-sling mechanism was more functionally disintegrated among post-treatment head and neck cancer patients than in the control group.

This thesis does not consider the role of the tongue or of infrahyoid muscles, except the thyrohyoid, in hyolaryngeal elevation. The movement of the tongue does interact with the hyolaryngeal complex. However, tongue movement is primarily associated with the oral phase and the initiation of the pharyngeal phase of swallowing (Hiiemae and Palmer,

2003). For the purpose of this thesis, it is assumed that the movement of the tongue affecting the posture of the hyolaryngeal complex is primarily associated with the oral phase of swallowing, and not hyolaryngeal elevation in the pharyngeal phase of swallowing. The omohyoid, sternothyroid, and sternohyoid muscles are thought to acts as antagonists of the two-sling mechanism to help posture the hyoid and larynx during the pharyngeal phase of swallowing. The activation of these infrahyoid muscles begins as the mylohyoid reaches peak activity (Thexton et al., 2007). It is assumed in this thesis that these infrahyoid muscles, and the mediastinal structures suspended by the hyolaryngeal complex, contribute to downward forces that must be overcome by the two-sling mechanism elevating the hyolaryngeal complex.

In summary, the following chapters will demonstrate the anatomical, functional, kinematic, and morphological evidence for a two-sling mechanism of hyolaryngeal elevation in the pharyngeal phase of swallowing. After establishing anatomical evidence for the potential roles of muscles in the two slings (Chapters 2 and 3), these muscles are then demonstrated to be active during swallowing (chapter 4). We then document the action of the muscle groups during swallowing (chapter 5). After demonstrating the action of these muscles we describe how the muscular and skeletal elements of the two slings function together as a mechanism to elevate components of the hyolaryngeal complex (chapter 6). The clinical relevance of the two-sling mechanism is explored by comparing a group of patients with poor swallowing function with a control group (chapter 7). The final study (chapter 8) documents how the musculoskeletal elements of the two slings function as covariant components of a system, and demonstrates the effect of pathology and aging on the functional integration of these components. The purpose of this series of investigations is to build an argument for the underlying mechanism of hyolaryngeal elevation in swallowing in order to improve the clinical assessment and treatment of dysphagia.

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Chapter 2

Evaluating the Structural Properties of Suprahyoid Muscles and Their Potential for Moving the Hyoid

Abstract

Introduction: Superior and anterior hyoid movements are important events in pharyngeal deglutition. This cross-sectional study uses a cadaver model to document the structural properties of the muscles underlying these movements in an effort to understand how their morphology influences function.

Methods: Measurements to determine physiological cross-sectional areas (PCSA) of swallowing muscles were taken from hemisected head and neck formalin fixed cadaver specimens (n=13 specimens). Coordinates of muscle attachment sites and PCSAs were used to calculate $\hat{\imath}$ and $\hat{\jmath}$ unit force vectors where $\hat{\imath}$ and $\hat{\jmath}$ represent anterior-posterior and superior-inferior directions, respectively. The suprahyoid muscle subsamples were grouped for analysis as follows: digastrics (DG), geniohyoid (GH), mylohyoid (MH), and stylohyoid (SH).

Results: The ANOVA with Tukey HSD post hoc analysis of unit force vectors showed the following results: GH (-0.44±.15 cm^2) > MH (-0.02±.21 cm^2), DG (-0.05±.11 cm^2), SH (0.14±.04 cm^2) with negative values representing the anterior direction ($p < 0.01$), and MH (0.91±.28 cm^2) > DG (.29±.14 cm^2), SH (0.22±.08 cm^2), GH (12±.08 cm^2) with positive values representing superior direction ($p < 0.01$).

Discussion: The morphology of the suprahyoid muscles suggests that based on structural properties, the geniohyoid has the most potential to displace the hyoid in the anterior direction and the mylohyoid has the most potential to displace the hyoid in the superior

direction. These data in complement with physiological findings may provide greater insight into these movements for those developing novel treatments for dysphagia.

Chapter 2

Evaluating the Structural Properties of Suprahyoid Muscles and Their Potential for Moving the Hyoid

Introduction

Prior to fluoroscopy and electromyography, the function of the anatomy underlying hyoid movement was poorly understood. Videofluoroscopy has since verified and quantified the superior and anterior movement of the hyoid (Dodds et al., 1990; Kim and McCullough, 2008; Leonard et al., 2000; Perlman et al., 1995) (Fig. 11). The suprahyoid muscles are the presumed muscles affecting this movement as shown in animal studies correlating electromyography and fluoroscopy (German et al., 2009). However, the structural properties of muscles determining their potential contribution to the superior and anterior movement of the hyoid are not documented. Understanding the structural contributions of individual muscles in the context of functional findings may have important clinical implications for surgeons concerned with retaining function, clinicians seeking to improve swallowing function with exercise regimens, and investigators hoping to augment function technologically.

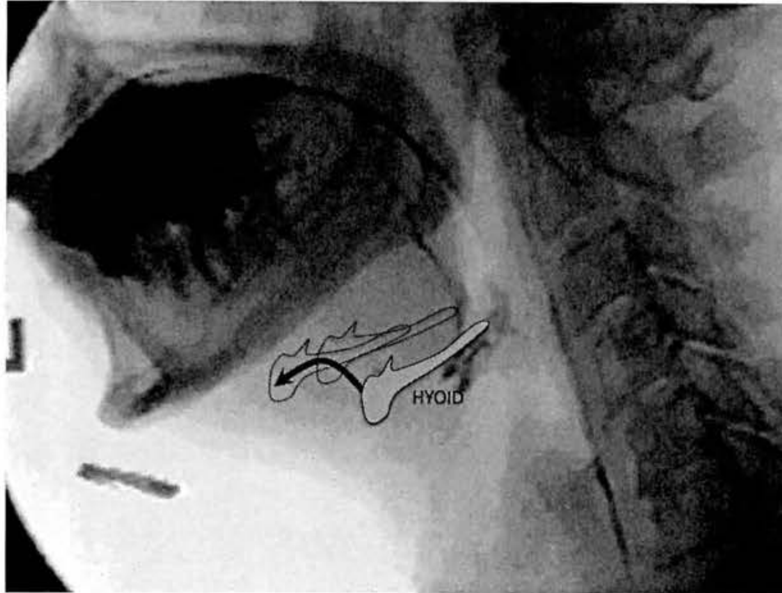

Fig. 11. Illustration of the superior and anterior movement of the hyoid bone as seen in fluoroscopy under normal conditions

Current swallowing theory suggests that the suprahyoid muscles, along with the thyrohyoid muscle, are primarily responsible for the opening of the upper esophageal sphincter (Matsuo and Palmer, 2008; Cook, 1989). Perlman et al, used electromyography to show that activation of the submental muscles (geniohyoid, mylohyoid and anterior digastric) correlates with the inhibition of the cricopharyngeus and the opening of the sphincter in humans (Perlman et al., 1999). Kurt et al. demonstrated that the stylohyoid and posterior digastric muscles as a complex are activated in human deglutition (Kurt et al., 2006). The purpose of the present investigation is to document the structural properties of the suprahyoid muscles (using human cadavers) to better understand their potential relative contributions to the movement of the hyoid.

The suprahyoid muscles include the geniohyoid, stylohyoid, mylohyoid, anterior digastric and posterior digastric (Fig. 12). The geniohyoid and stylohyoid originate from the

mandible and cranial base respectively. The mylohyoid, consisting of an anterior and posterior portion, arises from the mandible and inserts on a midline raphe and the body of the hyoid. The anterior and posterior belly of the digastric arise from the mandible and cranial base respectively and share a common tendon translating force to the hyoid through a fibrous band of connective tissue.

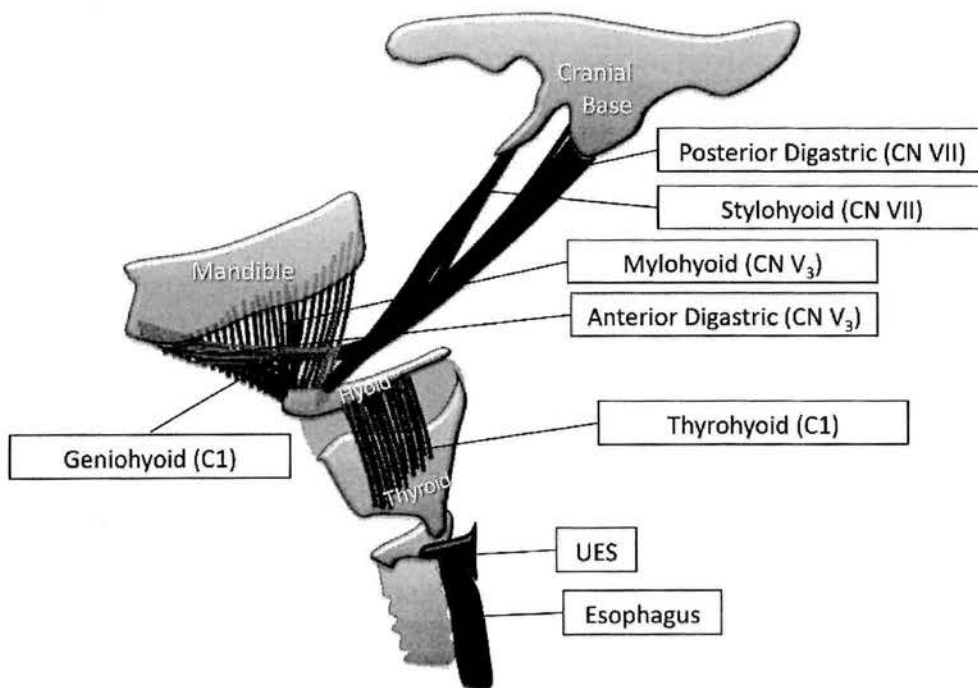

Fig. 12. Illustrated suprahyoid muscles with attachment sites and innervation. The suprahyoid and thyrohyoid muscles, among other functions, are thought to open the upper esophageal sphincter (UES).

It should be underscored that findings based on structure alone will not explain or predict what actually happens in nature. The composition of fiber types, passive forces of a

muscle, and motor units recruited for a specific task such as swallowing are essential to understanding function, and conclusions cannot be drawn from mere structural data. However, muscle function is constrained by its structural properties. What we can say is that based on structural data, muscles have the potential for certain actions and that these data may then be helpful in understanding the foundation of swallowing movements and treating swallowing dysfunction.

The actions of the muscles examined in the present study are described using force vectors, which describe the magnitude and direction of muscle pull. To isolate the potential structural contributions of muscles in the anterior and superior direction, unit force vectors are calculated for each muscle. This approach provides a quantitative way to evaluate the potential effect of individual muscles on a movable structure such as the hyoid (van Eijden et al., 1997). By calculating the unit force vectors for each of the suprahyoid muscles, we tested the hypotheses that structurally: (I) the geniohyoid has the most potential to affect the anterior displacement of the hyoid, and (II) the mylohyoid has the most potential to affect the superior displacement of the hyoid.

Methods

Subjects

The Boston University School of Medicine Anatomical Gifts program granted permission to conduct this research, which was in full compliance with the intent and permissions of their program. The Boston University Medical Campus Institutional Review Board holds that cadaveric research on bodies donated to Boston University School of Medicine anatomical gifts program is ethical and exempt from the Institutional Review Board process. Thirteen hemi-sections of head and neck formalin fixed cadaver specimens were selected for this study. Structural properties of the suprahyoid muscles, including the geniohyoid, stylohyoid, anterior digastric, posterior digastric and the anterior and posterior portion of the mylohyoid, were recorded.

Physiological Cross Sectional Area (PCSA)

Maximum potential magnitude of muscle pull is calculated using physiological cross sectional area (PCSA), which is a proxy for potential maximum tetanic tension (Lieber and Fridén, 2000). PCSA is calculated from muscle mass (g), pennation angle (Θ), fiber length (cm), and density (ϱ) as follows: (See Table 1)

$$PCSA = \frac{mass(g) \bullet \cos\Theta}{\rho(g/cm^3) \bullet FL_{optimal}(cm)}$$

Mass and Density

Muscles were harvested and soaked in 0.2M phosphate buffered saline for 48 hours according to a protocol that calibrates tissue density to 1.112 g/cm^3 (Ward and Lieber, 2005). Muscle mass was measured with a Mettler-Toledo PB602-S digital balance (Mettler-Toledo, Greifensee, Switzerland)

Pennation angles

Surface pennation angles of muscles were measured by photomacrographic digital image analysis. The digital photomacrograph workstation includes a photomacrography table (Polaroid, Cambridge, MA) with mounted 10-megapixel Canon EOS Rebel XTi digital camera with 35mm lens (Canon, Tokyo, Japan). Images were analyzed with Image J software (http://rsbweb.nih.gov/ij) and calibrated to a standard recorded on the image.

Fiber Lengths (FL)

PSCA calculations measure fiber length functionally as bundles of fibers or muscle fascicles. Fascicle bundles were separated with an Olympus OME surgical dissecting microscope at 2.5x magnification (Olympus, Tokyo, Japan) and measured using photomacrographic digital image analysis with Image J software (http://rsbweb.nih.gov/ij). Since muscles fix at either concentric or eccentric contraction, measured FLs were adjusted to optimal sarcomere length using the following equation to reflect maximum tension (Walker and Schrodt, 1974):

$$FL_{optimal} = FL(2.81\mu m/SL)$$

Sarcomere Lengths (SL)

Muscle tissue samples from distal, proximal and midpoints of muscle fascicles were wet mounted with glycerol to avoid tissue-altering processing (Kamibayashi and Richmond, 1998). Photomicrographs were captured under a 60x objective. Sarcomere lengths were measured by photomicrographic digital image analysis with an average length of ten sarcomeres in series reported. The digital photomicrograph workstation includes a Nikon Eclipse 80i microscope (Nikon, Lake Placid, NY) equipped with an Optronics MicroFire digital camera (Optronics, Goleta, CA) connected to a Dell Optiplex GX270 computer (Dell Computer Co., Round Rock, TX) equipped with Stereo Investigator 7 software (MicroBrightField Inc., Williston, VT). Images were analyzed with Image J software (http://rsbweb.nih.gov/ij) and calibrated to a calibration scale recorded on the image.

Direction of Force

Plotting and connecting the attachments of each muscle in three dimensions describes the lines of action of each muscle. Muscle attachments were marked with pins and photographed. Photomacrographic images of the medial and lateral surfaces of the bisected skull and attached swallowing apparatus generated x- and y-coordinates of muscle attachments by digital image analysis. Hemisections were pinned so that the inferior edge of the mandible and anterosuperior edge of the hyoid aligned with the x-axis, and the anterosuperior edge of the hyoid and thyroid notch aligned with the y-axis. Coordinates in two dimensions of muscle attachments of the sample are shown in Fig. 13. The z-coordinates were measured with a Mitutoyo digital caliper model No. CD-600 CSX (Mitutoyo Corp., Kanogawa, Japan).

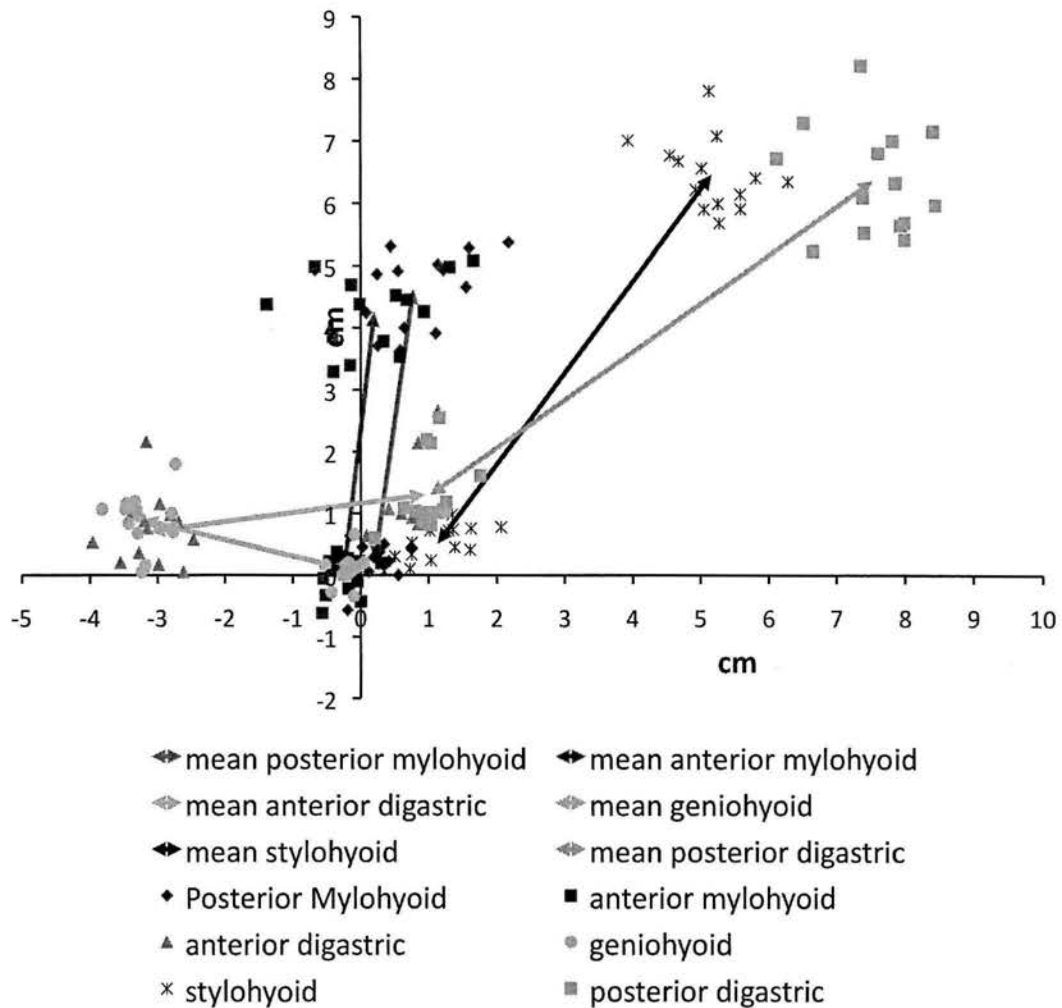

Fig. 13. Two-dimensional coordinates of muscle attachment points: Anterior = -x; Superior = +y; Hyoid = (0,0). Arrows represent a mean line of action for the indicated muscle sample (n=13).

Unit Force Vectors

Unit force vectors were calculated to isolate the potential contribution of each muscle to the relevant anatomical movements. The anterosuperior border of the hyoid was set as the origin. The $\hat{\imath}$ unit vectors were calculated from the x-coordinates to represent the anterior-posterior portion of a vector with anterior indicated in the negative direction ($-x$).

The $\hat{j}$ unit vectors were calculated from the y-coordinates to represent the superior-inferior portion of a vector with superior indicated in the positive direction $(+y)$.

$\hat{i} = \frac{x_2 - x_1}{d}$, representing the unit vector anterior-posterior direction

$\hat{j} = \frac{y_2 - y_1}{d}$, representing the unit vector superior-inferior direction

where, $d = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2 + (z_2 - z_1)^2}$.

The $\hat{i}$ or $\hat{j}$ unit vector multiplied by the PCSA as a proxy for force provides the potential maximum tension of a given muscle in the anterior-posterior or the superior-inferior directions:

$F_x = \hat{i} \times F$, representing the PCSA force unit vector anterior-posterior direction

$F_y = \hat{j} \times F$, representing the PCSA force unit vector superior-inferior direction

Statistics

Statistical tests used for these studies include a one-way analysis of variance with Tukey HSD post hoc analysis using web-based computer software: [Lowry, R. (1998-2010). VassarStats: Website for Statistical Computation [Computer software]. http://faculty.vassar.edu/lowry/VassarStats.html]

Results

The mean and standard deviation of PCSA values are as follows in cm^2: posterior portion of mylohyoid (0.43±.12), anterior portion of mylohyoid (0.82±.18), posterior digastric (0.64±.16), anterior digastric (0.55±.12), stylohyoid (0.27±.09), geniohyoid (0.46±.16) (Table 1).

Table 1. The means and standard deviations of structural properties[a] necessary to calculate the PCSA[b] (n=13; 7 males, 6 females)

	[a]Mass (g)	[a]Pennation Angles	[a]Fiber Length (cm)	[a]Sarcomere Lengths (μm)	[b]PCSA (cm^2)
Posterior Mylohyoid	2.17 ±.56	2.39±.85	4.75±3.92	2.95±.43	0.43±.12
Anterior Mylohyoid	3.03±.47	6.99±4.49	3.29±4.26	2.75±.34	0.82±.18
Posterior Digastric	2.53±.65	7.10±3.71	3.03±4.28	2.43±.51	0.64±.16
Anterior Digastric	2.37±.46	9.29±3.40	3.33±4.50	2.43±.30	0.55±.12
Stylohyoid	1.39±.46	5.02±1.83	4.69±7.11	2.80±.35	0.27±.09
Geniohyoid	2.21±.59	7.30±1.58	3.53±3.69	2.31±.55	0.46±.16

From three dimensional coordinates collected $\hat{\imath}$ unit vectors representing the anterior-posterior direction with $-\hat{\imath}$ representing anterior direction were calculated, the mean and standard deviations are as follows (all in cm^2): posterior portion of mylohyoid (0.09±.13), anterior portion of mylohyoid (-0.09±.21), posterior digastric (0.75±.06), anterior digastric (-0.95±.03), stylohyoid (0.53±.10), geniohyoid (-0.96±.03). The $\hat{\jmath}$ unit vectors representing the superior-inferior direction with $+\hat{\jmath}$ representing superior direction were calculated, the mean and standard deviations are as follows: posterior portion of mylohyoid (0.85±.05), anterior portion of mylohyoid (0.66 ±.18), posterior digastric (0.58±.06), anterior digastric (-0.15±.13), stylohyoid (0.80±.05), geniohyoid (0.24±.13). The $\hat{\imath}$ and $\hat{\jmath}$ unit vectors were multiplied times the PCSA values to calculate a PCSA force unit vector in the anterior-posterior and superior-inferior directions respectively. The anterior-posterior PCSA force unit vectors with $-\hat{\imath}$ representing anterior direction were calculated, the mean and standard deviations are as follows in cm^2: posterior portion of mylohyoid (0.04±.06), anterior portion of mylohyoid (-0.07±.18), posterior digastric (0.48±.12), anterior digastric (-0.53±.12), stylohyoid (0.14±.04), geniohyoid (-0.44±.15). The superior-inferior PCSA force unit vectors with $+\hat{\jmath}$ representing superior direction were calculated, the mean and standard deviations are as follows in cm^2: posterior portion of mylohyoid (0.37±.10), anterior portion of mylohyoid (0.55 ±.22), posterior digastric (0.38±.12), anterior digastric (-0.09±.08), stylohyoid (0.22±.08), geniohyoid (0.11±.07).

For the statistical analysis of PCSA force unit vectors the mylohyoid was considered to perform as a unitary muscle. The analysis of variance of the PCSA force unit vectors in the anterior-posterior direction for the mylohyoid (MH), posterior digastric (PDG), anterior digastric (ADG), stylohyoid (SH) and geniohyoid (GH) showed a statistically significant difference between all groups ($p<0.0001$). A Tukey HSD post hoc analysis showed that there were significant differences in the anterior-posterior PCSA force unit vector between each muscle sample except for the ADG and GH that are indicated to possess the greatest potential tension in the anterior direction (MH vs. PDG, $p=0.01$; MH vs. ADG, $p=0.01$; MH vs. SH, $p=0.05$; MH vs. GH, $p=0.01$; PDG vs. ADG, $p=0.01$; PDG vs. SH, $p=0.01$; PDG vs. GH, $p=0.01$; ADG vs. SH, $p=0.01$; SH vs. GH $p=0.01$). The literature reports that both the anterior and posterior digastrics are active in deglutition (Kurt et al., 2006; Perlman et al., 1999). Electromyography data alone does not specify if muscles are in eccentric or concentric contraction. For the purposes of morphological analysis, if it is assumed that in the overall process of deglutition the posterior and anterior digastrics concentrically contract in tandem to translate force to the hyoid bone through a fibrous loop to elevate the hyoid, then the digastric muscles counter balance each other in the anterior-posterior direction. Under these assumptions, the PCSA force unit vector for the geniohyoid is greater than all other muscles ($p=0.01$)(Fig. 14).

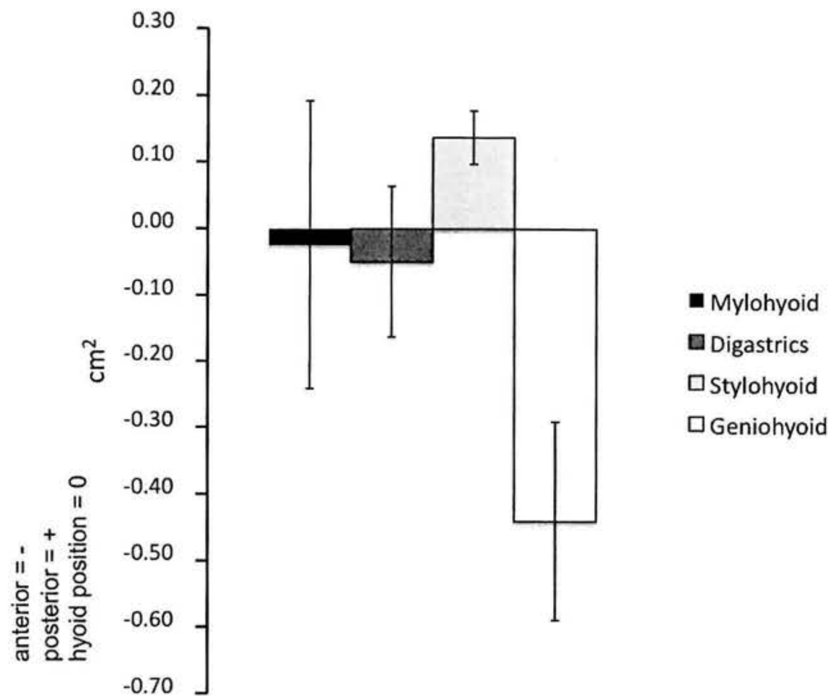

Fig. 14. Means and standard deviations of anterior-posterior PCSA force unit vectors (cm^2) with: anterior = - ; posterior = +; hyoid position = 0. An analysis of variance with Tukey HSD of the unit force vector in the anterior-posterior direction shows that the geniohyoid has the most potential structurally to effect the anterior displacement of the hyoid ($p < 0.01$).

The analysis of variance of the PCSA force unit vectors in the superior-inferior direction for the mylohyoid, posterior digastric, anterior digastric, stylohyoid and geniohyoid showed a statistically significant difference between all groups ($p<0.0001$). A Tukey HSD post hoc analysis showed that there were significant differences in the superior-inferior PCSA force unit vector between each muscle sample except for the PDG and SH and the SH and GH with the MH greater than all others. (MH vs. PDG, $p=0.01$; MH vs. ADG, $p=0.01$; MH vs. SH, $p=0.01$; MH vs. GH, $p=0.01$; PDG vs. ADG, $p=0.01$; PDG vs. GH, $p=0.01$; ADG vs. SH, $p=0.01$; ADG vs. GH, $p=0.05$). Even when it is assumed

that the posterior and anterior digastrics act in tandem to translate force to the hyoid bone, the mylohyoid is structured to translate a greater potential superior tension on the hyoid bone than the digastrics. In either case, the PCSA force unit vector in the superior direction for the mylohyoid is greater than all other muscles (p=0.01)(Fig. 15).

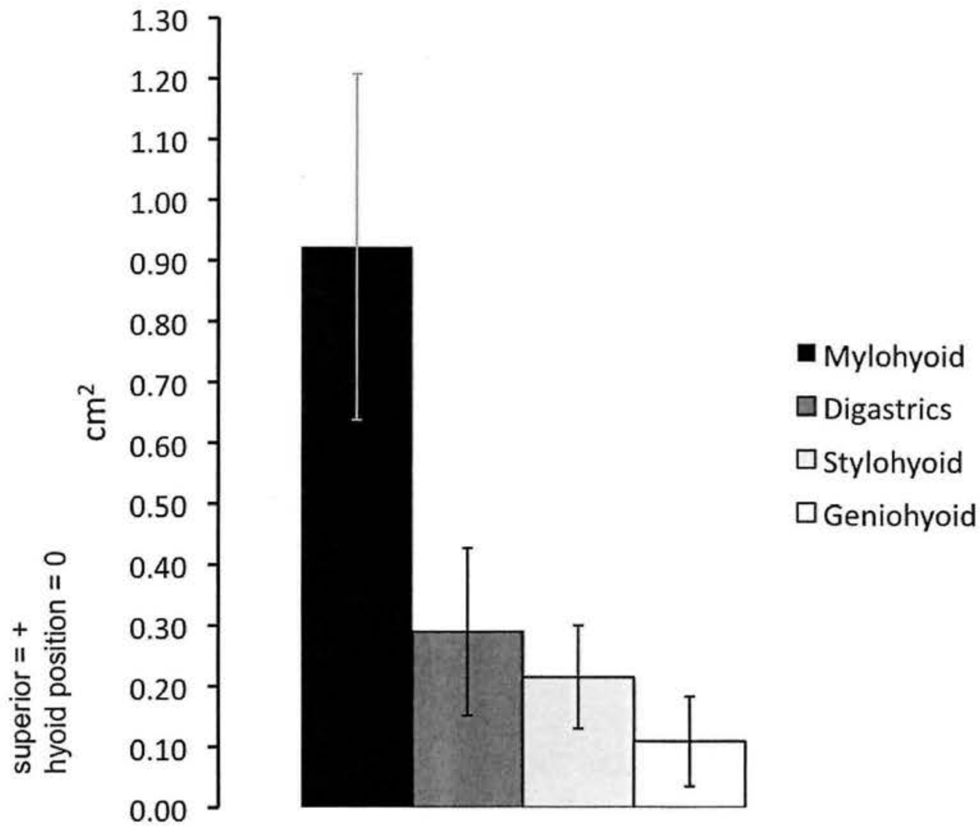

Fig. 15. Means and standard deviations of superior-inferior PCSA force unit vectors (cm^2) with: superior = +; hyoid position = 0. An analysis of variance with Tukey HSD of the unit force vector in the superior-inferior direction shows that the mylohyoid has the most potential structurally to effect the superior displacement of the hyoid (p<0.01).

Discussion

Understanding the potential relative contribution of each of the suprahyoid muscles may advance the treatment of dysphasia in several ways. Since the geniohyoid and mylohyoid have more structural potential for displacing the hyoid anteriorly and superiorly, respectively, these two muscles could be preferentially targeted for neuromuscular stimulation (Burnett et al., 2005). Studies have also shown that exercise can increase motor unit recruitment of muscles for particular functions (Robbins et al., 2008). By understanding the potential for hyoid excursion demonstrated by the structures of these muscles, therapists could target specific muscles with exercises designed to promote hyoid excursion. Additionally, awareness of the function of these muscles by radiation and surgical oncologists may reduce treatment-induced dysphagia (Feng et al., 2007; Kronenberger and Meyers, 1994). For example, greater efforts might be made during neck dissections to preserve the nerve to the mylohyoid or the C1 branch that supplies the geniohyoid. Finally, these structural data may be useful to those using computers to model deglutition.

Force vectors determined by PCSA measurements alone cannot predict function since motor unit recruitment of these multifunctional muscles will vary according to the task and stimuli. The pattern of recruitment likely varies when these muscles perform their various roles in deglutition, mastication, phonation or respiration. While structural properties do constrain the limits of function, they cannot be taken on their own to represent function. Instead, these data must be complemented by insight from various other studies. For example, fluoroscopy studies have shown that superior movement of the hyoid tends to precede anterior movement (Dodds et al., 1990; Leonard et al., 2000). Electromyography data in animal studies have shown that the activation of mylohyoid precedes the geniohyoid (German et al., 2009; Thexton et al., 2007). These results correlate nicely with the implications of the structural findings of the current investigation.

There are some potential limitations to the methods used in this study. The structural properties of non-contractile tissue such as tendon and fascia are not included in this study. The passive properties of these tissues may influence the position of the hyoid and be an important consideration for future studies. There is also some question as to the best way to generate PCSA data (Cutts and Seedhom, 1993). Some have suggested using MRI to assess PCSA *in vivo* using muscle volumes (Maganaris et al., 2001). However, this method at present cannot account for sarcomere lengths, which puts the reliability of the measure in question as a representation of maximum tetanic tension. Finally, the structural properties reported here were measured from a sample of subjects > 65 years of age. Estimates of swallowing difficulty range from 16-22% of patients over age 50 (Cook and Kahrilas, 1999). Therefore, we cannot assume that these individuals enjoyed normal swallowing during life. Therefore, the structural properties of these muscles may not be equivalent to those in individuals with normal swallowing in the general population, but may instead represent the structural properties of muscles in an elderly population.

Conclusions

The morphology of the suprahyoid muscles suggests that based on structural properties alone; the geniohyoid has the most potential to displace the hyoid in the anterior direction and the mylohyoid has the most potential to displace the hyoid in the superior direction.

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Chapter 3

Analyzing the Structure of Muscles Elevating the Hyolaryngeal Complex

Abstract

Introduction: A critical event of pharyngeal swallowing is the elevation of the hyolaryngeal complex, which protects the airway and opens the upper esophageal sphincter. Current swallowing theory assigns this function to the submental (geniohyoid, mylohyoid, and anterior digastric) and thyrohyoid muscles. However, the attachments of the long pharyngeal muscles (palatopharyngeus, salpingopharyngeus, stylopharyngeus) indicate that they could contribute to this function, yet their role is uninvestigated in humans. In addition, recent evidence suggests that suprahyoid muscles (submental muscles and posterior digastric and stylohyoid) act on the hyoid. A cadaver model is used to document the structural properties of muscles. These properties were used to model muscle groups as force vectors and analyze their potential for hyolaryngeal elevation.

Methods: Vector magnitude was determined using physiological cross-sectional areas (PCSAs) of muscles calculated from structural properties of muscle taken from twelve hemisected cadaver specimens. Vector direction (lines of action) was calculated from the three-dimensional coordinates of muscle attachment sites. Unit force vectors in the superior direction of submental, suprahyoid (which include the submental muscles), long pharyngeal, and thyrohyoid muscles were derived and compared by an analysis of variance to document each muscle's potential contribution to hyolaryngeal elevation.

Results: An ANOVA with Tukey HSD post hoc analysis of unit force vectors showed no statistically significant difference between the submental (0.92 ± 0.24 cm^2) and long pharyngeal (0.73 ± 0.20 cm^2) muscles. Both demonstrated greater potential to elevate the

hyolaryngeal complex than the thyrohyoid (.49±0.18 cm^2) with $p<0.01$ and $p<0.05$, respectively. The suprahyoid muscles (1.52±0.35 cm2) demonstrated the greatest potential to elevate the hyolaryngeal complex; greater than both the long pharyngeal muscles ($p<0.01$) and thyrohyoid ($p<0.01$).

Discussion: The submental and thyrohyoid muscles by convention are thought to elevate the hyolaryngeal complex. It should be noted the thyrohyoid is intrinsic to the complex and contributes to the movement of the larynx towards the hyoid. This study demonstrates that structurally the long pharyngeal muscles have similar potential to contribute to this critical function, with the suprahyoid muscles having the greatest potential. If verified by functional data, these findings would amend current swallowing theory.

Chapter 3

Structural Analysis of Muscles Elevating the Hyolaryngeal Complex

Introduction

A crucial event in the pharyngeal phase of swallowing is the elevation of the hyolaryngeal complex (Fig. 16) following bolus transfer into the pharynx and preceding pharyngeal constriction. Hyolaryngeal elevation occurs concomitant with the opening of the upper esophageal sphincter. This combination of movements displaces the larynx away from the trajectory of an oncoming bolus, shortens the pharynx, and pulls open the otherwise closed upper esophageal sphincter to receive the ingested bolus (Matsuo and Palmer, 2008). Reduced elevation of the larynx has been shown to be associated with aspiration (Bingjie et al., 2010). Submental muscles (mylohyoid, geniohyoid, and anterior digastric) and the thyrohyoid muscle are thought to elevate the hyolaryngeal complex in swallowing (Burnett et al., 2003; Ludlow et al., 2007) (Fig. 17). The hyolaryngeal complex includes the hyoid bone, thyrohyoid membrane, and laryngeal cartilages serving as attachment sites for the thyropharyngeus and cricopharyngeus muscles that form the upper esophageal sphincter. Other muscles attaching to the hyolaryngeal complex, including the posterior digastric, stylohyoid, and long pharyngeal muscles, have been named in the literature as potential contributors to this movement, but their roles have not been explicitly investigated (Cook et al., 1989; Kahrilas et al., 1992; Kurt et al., 2006). In a prior study a cadaver model was used to evaluate the architecture of muscles positioned to move the hyoid anteriorly and superiorly (Pearson et al., 2011, Chapter 2). The purpose of this study is to document the structural properties of muscles attaching to the hyolaryngeal complex and evaluate the potential contribution of various muscle groups in laryngeal elevation (Figs. 18,19).

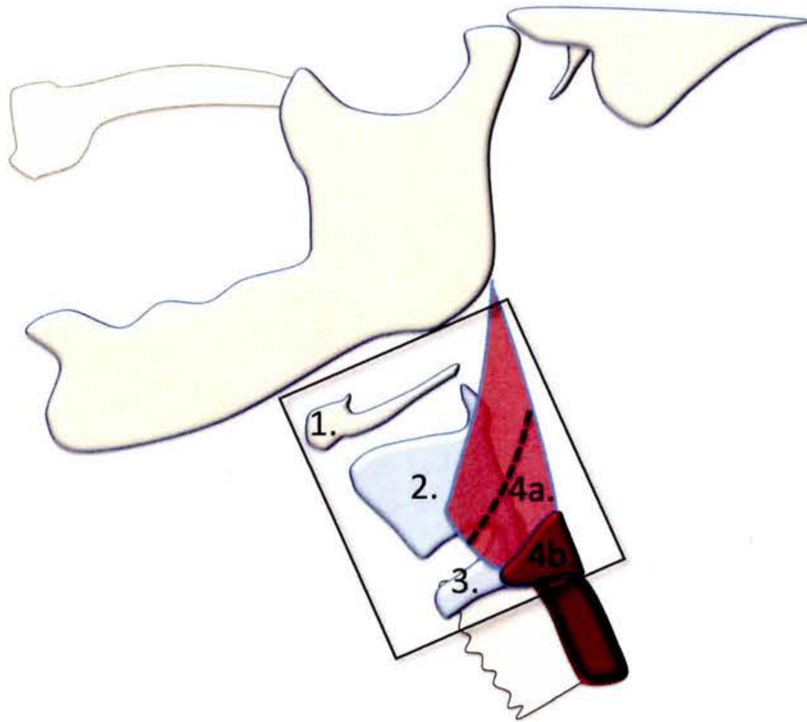

Fig. 16. Illustrated hyolaryngeal complex including 1.) hyoid bone, 2.) thyroid cartilage, 3.) cricoid cartilage, 4.) upper esophageal sphincter comprised of the a.) inferior portion of the thyropharyngeus and b.) cricopharyngeus.

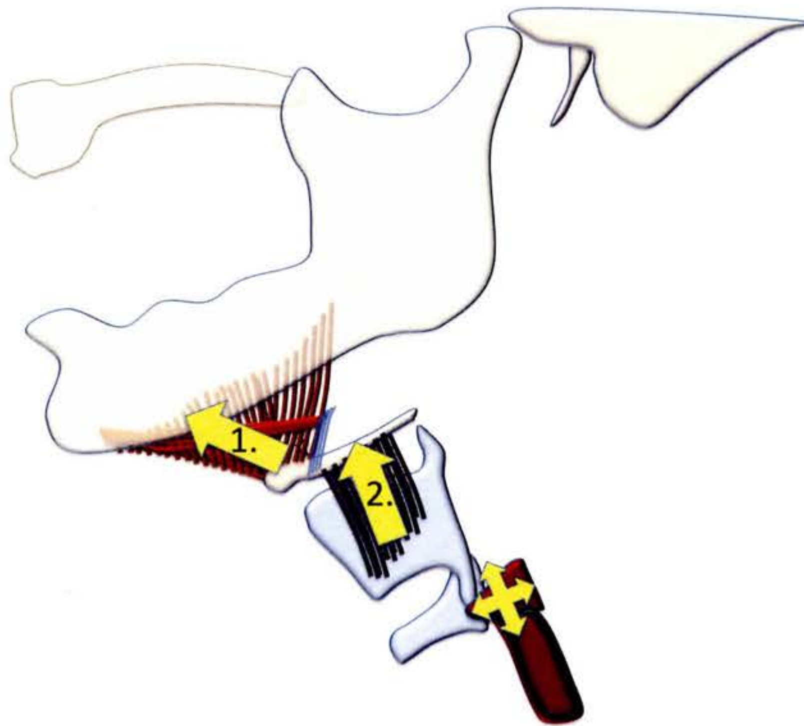

Fig. 17. Illustration of the current theory of hyolaryngeal elevation by the 1.) submental (mylohyoid, geniohyoid, anterior digastric) and 2.) thyrohyoid muscles. Some authors include the posterior digastric and stylohyoid in this model. This action is thought to open the upper esophageal sphincter.

We propose that anterior and posterior slings of muscles suspend the hyolaryngeal complex and elevate it in swallowing aided by the thyrohyoid (Figs. 18,19). The submental muscles attach to the hyoid (mylohyoid, geniohyoid, anterior digastric) and by convention are thought to form the anterior sling (Fig. 18). Additionally, the distal attachments of the remaining suprahyoid muscles, posterior digastric and stylohyoid, suggest that these muscles also contribute to the anterior sling (Kurt et al., 2006; Thexton et al., 2007) (Fig. 19). The long pharyngeal muscles, which include the stylopharyngeus,

salpingopharyngeus, and palatopharyngeus stabilized by the levator veli palatini, form a posterior sling. The distal attachments of these muscles blend together on the posterior edge of the thyroid cartilage and in the lateral pharyngeal wall proximal to the upper esophageal sphincter (Okuda et al., 2008). Finally, the thyrohyoid muscle is intrinsic to the hyolaryngeal complex and approximates the thyroid and hyoid. These muscle groups are summarized in Fig. 19.

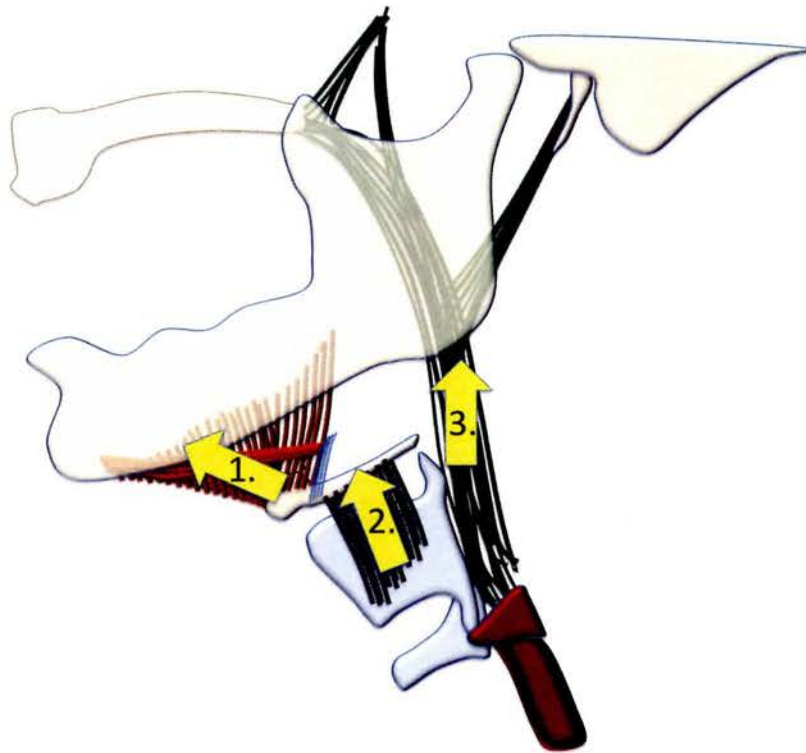

Fig. 18. Illustration of the two-sling theory with the 1.) submental muscles as an anterior sling, the 2.) thyrohyoid muscle and the 3.) long pharyngeal muscles (stylopharyngeus, palatopharyngeus, and salpingopharyngeus) as a posterior sling.

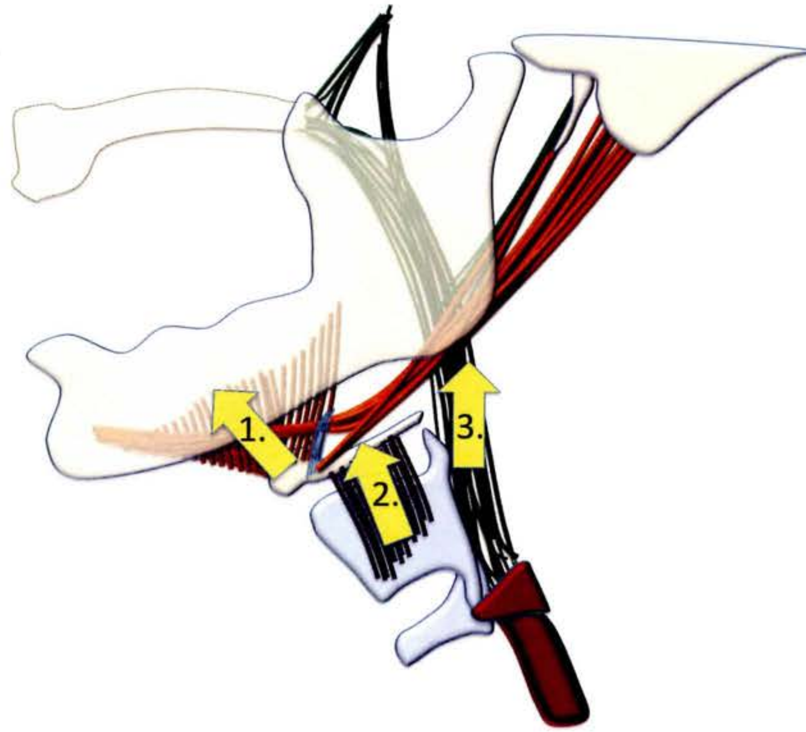

Fig. 19. Illustration of the two-sling theory with the 1.) suprahyoid muscles group (submental, posterior digastric, and stylohyoid) as an anterior sling, the 2.) thyrohyoid muscle and the 3.) long pharyngeal muscles as a posterior sling.

To test the potential contribution of the submental group, suprahyoid (submental + posterior digastric and stylohyoid) group, long pharyngeal group and thyrohyoid muscle to hyolaryngeal elevation, we modeled each muscle as a potential force vector based on structural properties and used these data to analyze the potential contribution of the functional muscle groups to elevation of the hyolaryngeal complex (Figs. 18,19). It should be underscored from the start that structural properties are not necessarily the same as functional realities. It is unlikely that force vectors based on morphology alone are entirely predictive of swallowing function (German et al., 2011). Structural analysis

of muscles in a cadaver can say nothing about the motor unit recruitment that ultimately determines the relative contribution of muscles *in vivo*. However, neuromuscular control of swallowing is constrained by muscular architecture. This reconsideration of the potential underlying structure of hyolaryngeal elevation is important since if the usual list of muscles assumed to underlie a swallowing function is incomplete, then a function may be erroneously attributed to the wrong muscles.

This study investigates structural properties to document the anatomical substrate underlying this critical event in pharyngeal deglutition. We propose that two muscular slings along with the thyrohyoid may elevate the hyolaryngeal complex. Our supposition is that the long pharyngeal muscles have as much structural potential to elevate the hyolaryngeal complex as the submental or thyrohyoid muscles. We predict that the PCSA force vector analysis of these muscle groups will show no statistically significant difference between the long pharyngeal muscles and submental muscles, and that the suprahyoid muscles as a group have a greater potential for hyolaryngeal elevation than all other groups.

Methods

Subjects

The Boston University School of Medicine Anatomical Gifts program granted ethical permission for this research in compliance with the Institutional Review Board and the wishes of body donors. After specimens were used for educational purposes, seven partially dissected bodies were chosen for this study. Of the possible fourteen head and neck formalin fixed hemisections, two were not fully intact and excluded.
Muscles included for evaluation in the present study are: geniohyoid, anterior digastric, mylohyoid, posterior digastric, stylohyoid, thyrohyoid, stylopharyngeus, palatopharyngeus and salpingopharyngeus. Values for the geniohyoid, anterior digastric, mylohyoid, posterior digastric, and stylohyoid were taken from a previous study (chapter 2). Values for the thyrohyoid, stylopharyngeus, palatopharyngeus and

salpingopharyngeus were newly derived. The stylopharyngeus was analyzed in its two parts: the superior portion inserting into the lateral pharyngeal wall and the inferior portion inserting into the posterior edge of the thyroid cartilage. This distinction was made based on our consistent observation of these features. The salpingopharyngeus and the palatopharyngeus were measured as one muscle since their fibers are blended except for their superior attachment sites.

The muscles were grouped in two configurations to analyze the potential of muscle groups to elevate the hyolaryngeal complex. One configuration reflected current theory and compared the long pharyngeal muscles (stylopharyngeus, palatopharyngeus and salpingopharyngeus) to the submental muscles (mylohyoid, geniohyoid, and anterior digastric) and the thyrohyoid. A second configuration added the posterior digastric and the stylohyoid to the submental group to form a suprahyoid group of muscles. While it is quite likely that each of these muscles can act independently and is under separate neural control, our question related to the potential net effect of these muscles in the superior direction.

Vectors

Methods for modeling muscles as vectors to compare their potential for force in a particular direction are thoroughly described in a previous study of the suprahyoid muscles (Chapter 2). Briefly, physiological cross sectional area (PCSA) of each muscle, a proxy for the potential maximum tetanic tension the muscle is capable of producing, is utilized as a measure of vector magnitude (Lieber and Fridén, 2000; van Eijden et al., 1997). The PCSA calculation uses muscle mass (g) and density (ϱ) and muscle fiber pennation angles (θ) and optimized fascicle lengths (cm) to calculate the functional cross sectional area of the muscle, in cm^2.

$$PCSA = \frac{mass(g) \bullet \cos\Theta}{\rho(g/cm^3) \bullet FL_{optimal}(cm)}$$

Muscle tissue samples from distal, proximal and midpoints of muscle fascicles were wet mounted with glycerol to avoid tissue-altering processing (Kamibayashi and Richmond,

1998). Photomicrographs of muscle fascicles were captured under 60x magnification. The digital photomicrograph workstation included a Zeis Axioskop microscope (Zeis, Oberkochen, Germany) equipped with an Olympus Q-Colors 5 digital camera (Olympus, Center Valley, PA) connected to a Dell Precision T5400 computer (Dell Computer Co., Round Rock, TX) equipped with QCapture version 2.98.0 software (Quantitative Imaging Corporation, Surrey, BC). Images were analyzed with Image J software (ImageJ, National Institutes of Health, Bethesda, MD). Ten sarcomeres in series were measured from the middle of the I-band to the middle of the adjacent I-band using ImageJ dynamic profiler plug-in to calculate an optically precise average sarcomere length. An important step of proper PCSA calculation is to adjust mean fascicle lengths to their optimum length using a ratio of optimal sarcomere length to mean sarcomere lengths in the sample (Felder et al., 2005). In this study we tested a new method of measuring sarcomere lengths of thyrohyoid and long pharyngeal muscles using an ImageJ plug-in called dynamic profiler. We measured sarcomere lengths twice on a sample of palatopharyngeus muscles (n=8), once using the dynamic profiler to set the measure of the length tool and once without. A t-test demonstrated that there was no significant difference in its accuracy (p=0.48). Because the dynamic profiler allowed for a more efficient identification of boundaries between sarcomeres, this method was used for all muscles in this study.

Three-dimensional coordinates of the muscle attachments were recorded using digital image analysis of hemisections to generate x- and y-coordinates of muscle attachments. A handheld Mitutoyo digital caliper model No. CD-600 CSX (Mitutoyo Corp., Kanogawa, Japan) was used to measure z-coordinates. Vector directions were derived from the muscle lines of action, which were derived from connecting the three-dimensional coordinates of muscle attachments (Pearson et al., 2011). The potential for each muscle to create movement in the superior direction was derived for each muscle by isolating the portion of each unit vector in the superior direction. By multiplying PCSA values by the $\hat{j}$ (superior-inferior) unit vector of that muscle, we calculated PCSA force

unit vectors that represent the potential maximum tetanic tension of each muscle in the superior direction. These vectors were summed to form the functional groups described above and analyze the potential contribution of each muscle group to elevate the hyolaryngeal complex.

Analysis

We compared muscle groups using one-way analyses of variance with Tukey HSD post hoc analysis performed using VassarStats website for statistical computation (http://faculty.vassar.edu/lowry/VassarStats.html). To investigate if structurally the long pharyngeal muscles have the equivalent potential to elevate the hyolaryngeal complex as do the submental or thyrohyoid muscles, we compared the vertical (superior-inferior) PCSA force vectors of the long pharyngeal muscles with those of the submental muscles (geniohyoid, mylohyoid, and anterior digastric) and the thyrohyoid. A second analysis was conducted comparing vertical PCSA vectors of the long pharyngeal muscles to the suprahyoid muscles (geniohyoid, mylohyoid, anterior digastric, posterior digastric and stylohyoid) as a group and to thyrohyoid.

Results

The means and standard deviations of PCSA values calculated from structural properties were as follows, in cm^2: posterior portion of mylohyoid (0.43±.12), anterior portion of mylohyoid (0.82±.18), posterior digastric (0.64±.16), anterior digastric (0.55±.12), stylohyoid (0.27±.09), geniohyoid (0.46±.16), thyrohyoid (0.51±.18), stylopharyngeus (pharyngeal insertion) (0.12±.06), stylopharyngeus (thyroid insertion) (0.19±.07), palatopharyngeus and salpingopharyngeus (0.48±.17) (Table 2). The means and standard deviations of the PCSA force unit vectors in the superior direction were as follows, in cm^2: posterior portion of mylohyoid (0.85±.05), anterior portion of mylohyoid (0.66 ±.18), posterior digastric (0.58±.06), anterior digastric (-0.15±.13), stylohyoid (0.80±.05), geniohyoid (0.24±.13), thyrohyoid (0.49±.18), stylopharyngeus (pharyngeal insertion) (0.08±.04), stylopharyngeus (thyroid insertion) (0.18±.07), palatopharyngeus and

salpingopharyngeus (0.48±.17). To reiterate, data for the suprahyoid muscles was taken from Chapter 2.

Table 2. The means and standard deviations of structural properties[a] necessary to calculate the PCSA[b] for the thyrohyoid and long pharyngeal muscles (n=12; 6 males, 6 females). Previously reported data from chapter 2[1].

	[a]Mass (g)	[a]Pennation Angles	[a]Fiber Length (cm)	[a]Sarcomere Lengths (μm)	[b]PCSA (cm^2)
Thyrohyoid	1.56±.49	3.78±1.47	3.07±.66	2.90±.24	0.51±.18
Stylopharyngeus (pharyngeal insertion)	0.54±.26	3.92±1.57	4.05±.77	2.76±.40	0.12±.06
Stylopharyngeus (thyroid insertion)	1.35±.53	2.89±1.20	6.40±1.56	2.62±.48	0.19±.07
Palatopharyngeus/ Salpingopharyngeus	4.01±.75	3.53±1.27	8.73±2.14	2.80±.36	0.48±.17
Posterior Mylohyoid[1]	2.17 ±.56	2.39±.85	4.75±3.92	2.95±.43	0.43±.12
Anterior Mylohyoid[1]	3.03±.47	6.99±4.49	3.29±4.26	2.75±.34	0.82±.18
Posterior Digastric[1]	2.53±.65	7.10±3.71	3.03±4.28	2.43±.51	0.64±.16
Anterior Digastric[1]	2.37±.46	9.29±3.40	3.33±4.50	2.43±.30	0.55±.12
Stylohyoid[1]	1.39±.46	5.02±1.83	4.69±7.11	2.80±.35	0.27±.09
Geniohyoid[1]	2.21±.59	7.30±1.58	35.32±3.69	2.31±.55	0.46±.16

Comparison of the muscle groups (summed PCSA force unit vectors in the superior direction) showed no statistically significant difference between the submental (0.92±0.24 cm^2) and long pharyngeal groups (0.73±0.20 cm^2), though both were significantly larger than that for the thyrohyoid (0.49±0.18 cm^2) with $p<0.01$ and $p<0.05$ respectively (Fig. 20a). Comparison of the suprahyoid group, long pharyngeal and

thyrohyoid muscles showed the superior force vector of the suprahyoid group (1.52±0.35 cm^2) to be greater than that of the long pharyngeal ($p<0.01$) and thyrohyoid ($p<0.01$), and that of the long pharyngeal greater than that of the thyrohyoid ($p<0.05$)(Fig. 20b).

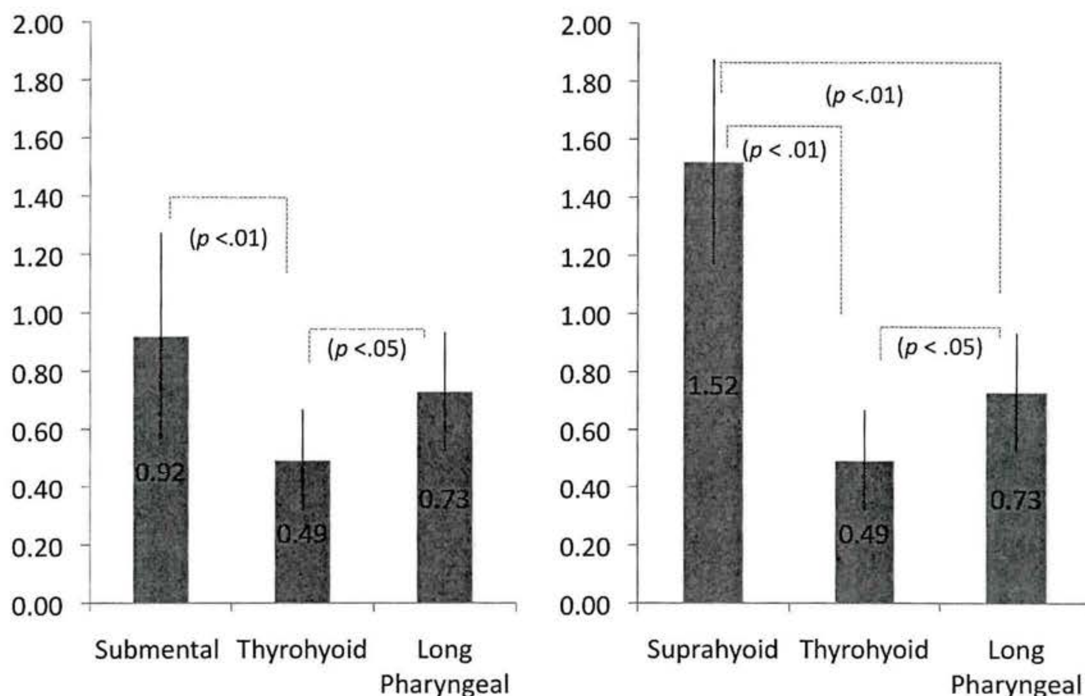

Fig. 20a-b. Means and standard deviations of superior PCSA force unit vectors (cm^2) of: submental vs. thyrohyoid vs. long pharyngeal muscles in Fig. 20a; and of suprahyoid vs. thyrohyoid vs. long pharyngeal muscles in Fig. 20b. An analysis of variance with Tukey HSD of the unit force vector shows that the long pharyngeal muscles have as much potential to elevate the hyolaryngeal complex as the submental muscles, though the suprahyoid muscles as a group have the greatest potential for force in hyolaryngeal elevation.

Discussion

The submental muscles and thyrohyoid are thought to underlie hyolaryngeal elevation opening the upper esophageal sphincter. The results of this study suggest that the proposed anterior and posterior muscular slings have the structural potential to synergistically elevate the hyolaryngeal complex during pharyngeal swallowing. The anterior sling muscles, which attach to the anterior portion of the hyoid, may conceptually include the submental muscles (mylohyoid, geniohyoid, and anterior digastric)(Fig. 18) or suprahyoid muscles (submental muscles plus stylohyoid and posterior digastric)(Fig. 19). The posterior sling, formed by the long pharyngeal muscles, attaches to the length of the posterior edge of the thyroid cartilage and the lateral pharyngeal walls in the hypopharynx. The results of this study indicate no statistically significant difference in the potential for force between the submental muscles as an anterior sling and the long pharyngeal muscles as a posterior sling. However, if the anterior sling is defined to include all of the suprahyoid muscles, these muscles have a statistically significant greater potential for force than do the long pharyngeal muscles. The attachments of the thyrohyoid muscle, analyzed separately from either sling in our model, indicate that it also aids in laryngeal elevation. If either of these two-sling models is verified in functional studies, this would amend current swallowing theory, which currently does not acknowledge the potential role of the long pharyngeal muscles.

To date there are no functional data to confirm the activity and action of all the long pharyngeal muscles in human swallowing. However, there is indication of palatopharyngeus involvement from animal and human studies. Electromyography data from a porcine model verify activity of the palatopharyngeus during pharyngeal swallowing in that animal (German et al., 2009; Thexton et al., 2007). Transection of the pharyngeal branches of the vagus nerve in a rabbit model results in diminished rostral movement of the larynx, a functional deficit that could be attributed in part to the loss of the long pharyngeal muscles (Fukushima et al., 2003). In human studies,

palatopharyngeus activity has been recorded using hookwire electrodes (Van Daele et al., 2005).

In a clinical study, Aydogdu et al. (Aydogdu et al., 2001) documented an apparent relationship between diminished neurologic control of these muscles and dysphagia. These researchers investigated the effects of Wallenburg's Syndrome in twenty human subjects, 95% of whom had clinical signs of dysphagia. In this syndrome, infarction of the lateral medulla affects a number of cranial nerve nuclei. One of these nuclei is the nucleus ambiguus, which provides motor innervation to the long pharyngeal muscles, among other important swallowing muscles. The same syndrome does not affect the nuclei for cranial nerves V and VII, which innervate the mylohyoid, digastrics and stylohyoid. In that study, subjects with Wallenburg's Syndrome demonstrated prolonged submental muscle activity during swallowing. One interpretation of this study may be that the anterior sling muscles compensate for the loss of posterior sling muscles.

Our structural observations provide some evidence that the current understanding of hyolaryngeal approximation is likely to be incomplete. If the usual list of muscles is assumed, the thyrohyoid is solely responsible for approximating the larynx to the hyoid bone (Cook et al., 1989; Mepani et al., 2009). Population data of mean hyolaryngeal approximation distances in humans across bolus sizes is 1.09 ±0.57cm in females and 1.29 ±0.47cm in males (Leonard et al., 2000). Muscle fiber at optimal length is shown to maximally contract by as much as 28 percent (Griffiths, 1991). In our cadaver sample, the mean optimal fascicle length of rehydrated thyrohyoid muscle was 2.77 ±0.58cm in females and 3.04 ±0.75cm in males (Ward and Lieber, 2005). Maximum thyrohyoid contraction in our sample would theoretically only result in thyrohyoid approximation of 0.78cm in females and 0.85cm in males. These structural analyses indicate that as much as 33% of laryngeal elevation may be attributed to the actions of other muscles that attach to the hyolaryngeal complex besides the thyrohyoid. Specifically, the inferior

attachments of the stylopharyngeus and palatopharyngeus are below the level of the hyoid bone, and could therefore move the larynx towards the hyoid.

The anterior sling muscles are oriented to execute anterior and superior displacement of the hyolaryngeal complex (Chapter 2). While their structure indicates that they can contribute more force to elevation of the hyolaryngeal complex, this does not necessarily mean they are more essential to hyolaryngeal elevation. As reflected in the calculation of PCSA, there is an inverse relationship between muscle force and fiber length. While the suprahyoid muscles have greater potential for *force*, the long pharyngeal muscles may be more responsible for laryngeal *excursion* based on their length advantage (Fig. 21). Further functional studies are needed to more thoroughly elucidate the relationship between structure and function in these muscles.

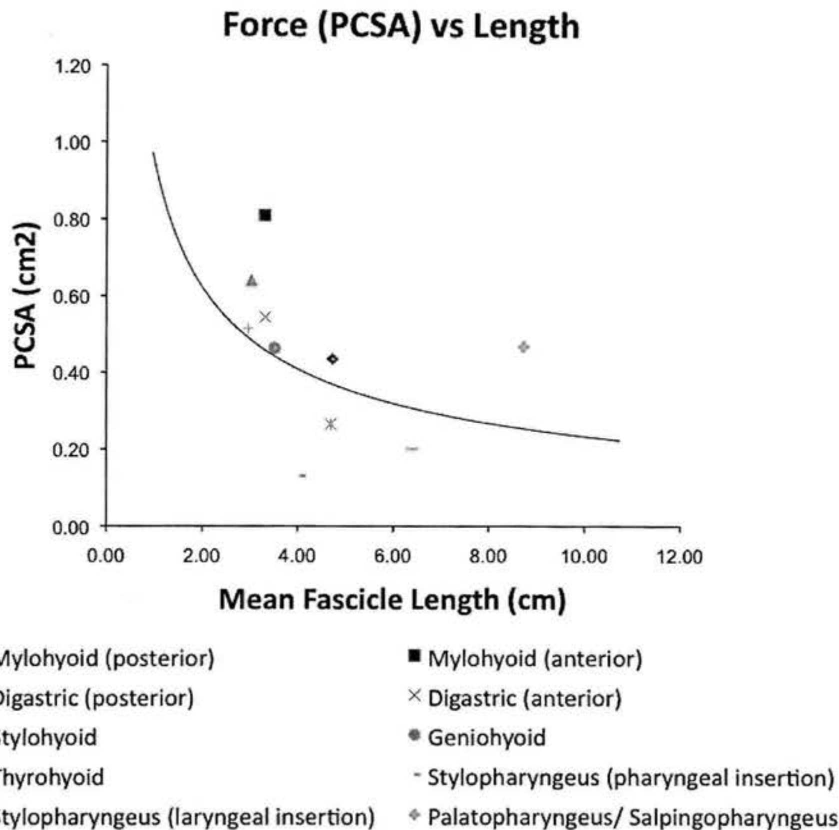

Fig. 21. Plot of mean PCSA (cm^2) vs. mean fascicle length (cm) of muscles included in this study with trend line indicating an inverse relationship between the potential for maximum tetanic tension and the potential for excursion. While in general the long pharyngeal muscles demonstrate less potential for force, they show greater potential for excursion given their length advantage.

A limitation of the current study is the exclusion of the pharyngeal constrictor and hyoglossus muscles. These muscles do not lend themselves to this method of force vector analysis: a line of action cannot represent the complex morphology of the pharyngeal constrictors, and the hyoglossus lacks a fixed superior attachment site. Obliquely oriented fibers of the middle and inferior pharyngeal constrictors attaching to the hyoid and thyroid cartilage may elevate the hyolaryngeal complex. However, it may also be that the mechanical advantage of obliquely oriented fibers of the pharyngeal

constrictors is nullified by hyolaryngeal elevation occurring prior to pharyngeal constriction (Kahrilas et al., 1992). The hyoglossus could contribute to hyoid elevation when the tongue is stabilized. The attachment of the hyoglossus-palatoglossus complex to the palatine aponeurosis may be functionally relevant to tongue placement or to hyolaryngeal elevation during bolus transit through the hypopharynx. Interestingly, it has been noted that following a complete glossectomy including the hyoglossus, patients can still safely swallow a bolus if the larynx is elevated (McConnel and O'Connor, 1994). While this limitation takes nothing away from the present findings, it does leave the structural contribution of these muscles to hyolaryngeal elevation as an open question. Three-dimensional computer modeling of digitized muscle fiber bundles may provide a method with which to investigate the morphology of these muscles in the future (Ravichandiran et al., 2009).

Another limitation that should be noted is that the swallowing function of the subject population was unverified before death. More importantly, it should be reiterated that structural properties are not the same as functional realities. Anatomical data, while suggestive of function, must be corroborated by functional studies to establish their clinical usefulness. We propose that the anterior and posterior muscular sling elevates the hyolaryngeal complex aided by the thyrohyoid muscle. If supported by functional data, this would change the way hyolaryngeal elevation during the pharyngeal phase of swallowing is conceptualized.

Conclusion

The morphology of the long pharyngeal muscles suggests that potentially they have a significant role in working with the thyrohyoid and submental muscles to elevate the hyolaryngeal complex during swallowing.

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Chapter 4

Evaluating Swallowing Muscles Essential to Hyolaryngeal Elevation with mfMRI

Abstract

Introduction: Reduced hyolaryngeal elevation, a critical event in swallowing, is associated with radiotherapy of the head and neck. A two-sling mechanism for hyolaryngeal elevation has been proposed: an anterior sling comprised of suprahyoid muscles, and a posterior sling comprised of the longitudinal pharyngeal muscles. Assisting both slings is the thyrohyoid, a muscle intrinsic to the hyolaryngeal complex. Intensity-modulated radiotherapy guidelines designed to preserve structures important to swallowing, currently exclude the suprahyoid and thyrohyoid muscles. This study uses muscle functional MRI in normal healthy adults to determine if both muscular slings are active in swallowing and to test the specificity of therapeutic exercises to target these muscles.

Methods: Muscle functional MRI (mfMRI) data were acquired from eleven healthy subjects before and after normal swallowing and after swallowing exercise regimens (Mendelsohn maneuver and the effortful pitch glide). Whole muscle transverse relaxation time (T2, measured in ms) profiles of 7 test muscles were used to evaluate physiologic response by each muscle to each condition. Effect size changes (Cohen's *d*) of whole muscle T2 profiles were used to determine which muscles underlie swallowing and swallowing exercises.

Results: Post-swallowing effect size changes for T2 signal profiles of muscles were as follows (Cohen's d>0.20 indicates a significant difference in physiological response to swallowing): thyrohyoid (d=0.09); anterior sling muscles: mylohyoid (d=0.40), geniohyoid (d=0.80), anterior digastric (d=0.04), posterior digastric/stylohyoid (d=0.25); and posterior sling muscles: palatopharyngeus (d=0.47) and stylopharyngeus (d=0.28).

The Mendelsohn maneuver (MM) and effortful pitch glide (EPG) swallowing exercises showed significant effect size changes for all muscles tested except thyrohyoid.

Discussion: Both muscular slings are active in swallowing and both swallowing exercises effectively target posterior sling muscles. Muscle functional MRI is useful in testing swallowing muscle function.

Chapter 4

Evaluating Swallowing Muscles Essential to Hyolaryngeal Elevation with mfMRI

Introduction

Radiation induced swallowing dysfunction is a widely recognized comorbidity associated with head and neck cancer treatment (Rosenthal et al., 2006). The mechanism of swallowing is extremely complex and not fully understood, however, post-radiotherapy dysphagia is most commonly attributed to reduced hyolaryngeal elevation and subsequent inadequate opening of the upper esophageal sphincter (Pauloski et al., 2006). Intensity-modulated radiotherapy (IMRT) promotes tissue sparing by shaping doses of radiation to avoid salivary glands and oropharyngeal structures thought essential to swallowing where possible (Eisbruch et al., 2004). Current IMRT recommendations focus on sparing the pharyngeal constrictor muscles and the larynx, but exclude muscles potentially important to hyolaryngeal elevation (Caglar et al., 2008; Christianen et al., 2011; Eisbruch et al., 2004; Levendag et al., 2007). The purpose of this study is to advance the current understanding of muscles essential to hyolaryngeal elevation in normal swallowing and explore how these findings compare with current IMRT guidelines.

The elevation of the hyolaryngeal complex is central to the intricate set of movements required to transfer a bolus from the oral cavity through the hypopharynx and into the esophagus. The hyolaryngeal complex consists of the hyoid, laryngeal cartilages, and muscles intrinsic to the hyoid and larynx including the thyrohyoid. The cricopharyngeus, the most inferior portion of the pharyngeal constrictor muscles, attaches to the hyolaryngeal complex and forms the upper esophageal sphincter (Matsuo and Palmer, 2008). Proper hyolaryngeal elevation draws the airway anterior to the trajectory of an oncoming bolus and stretches open a relaxed upper esophageal sphincter. Inadequate hyolaryngeal elevation can result in aspiration or bolus retention, putting the patient at risk for inadequate oral intake, need for an altered diet, and pneumonia.

Anatomical research has determined that morphologically, two extrinsic muscle groups have the greatest potential for hyolaryngeal elevation: an anterior muscular sling comprised of the mylohyoid, geniohyoid, anterior digastric, posterior digastric and stylohyoid muscles (the suprahyoid muscles), and a posterior muscular sling comprised of the stylopharyngeus, salpingopharyngeus, and palatopharyngeus muscles (the longitudinal pharyngeal muscles) (Pearson et al., 2012) (Fig. 22). In addition, the thyrohyoid muscle is thought to assist in approximating the larynx and hyoid. However, there is an apparent controversy in the literature. Of these muscles, current IMRT parameters protect the posterior sling muscles (excepting their proximal attachments) but not the anterior sling (suprahyoid) muscles or thyrohyoid (Christianen et al., 2011; Eisbruch et al., 2004). These exclusions are based on structural and not physiological findings (Eisbruch et al., 2004). Studies conducted in other clinical fields (speech-language pathology, otolaryngology, gastroenterology) argue that the suprahyoid (anterior muscular sling) and thyrohyoid muscles are primarily responsible for hyolaryngeal elevation, and therefore the opening of the upper esophageal sphincter (Cook et al., 1989). While anatomical studies propose the idea that both slings are important for hyolaryngeal elevation (and thus resolving the controversy) the functional data verifying these muscles are active in swallowing is incomplete.

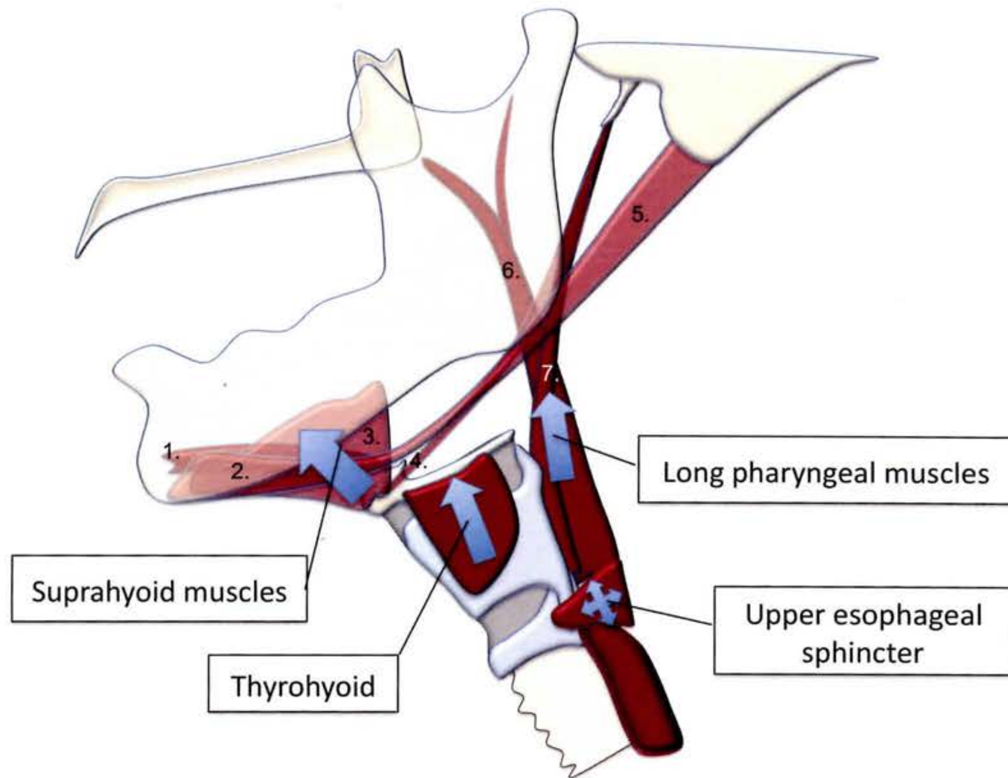

Fig. 22. Schematic of the two-sling mechanism for hyolaryngeal elevation in swallowing. This mechanism stretches open an inhibited cricopharyngeus, which forms the upper esophageal sphincter. The suprahyoid muscles (1. geniohyoid, 2. anterior digastric, 3. mylohyoid, 4. stylohyoid, 5. posterior digastric) comprise the anterior sling and the long pharyngeal muscles (6. palatopharyngeus, 7. stylopharyngeus) a posterior muscular sling. The thyrohyoid muscle is intrinsic to the hyolaryngeal complex and approximates the larynx and hyoid synergistically with the long pharyngeal muscles.

The muscles of interest in this study have not all been verified as active during swallowing with electromyography. The most comprehensive electromyography study of human swallowing did not include the stylopharyngeus, thyrohyoid, or digastric muscles (Van Daele et al., 2005). Deep muscles such as the stylopharyngeus are inaccessible to

electrodes. Muscle functional magnetic resonance imaging (mfMRI) overcomes this limitation by allowing for a semi-quantitative measurement of muscle activity after an episode of muscle use (Segal, 2007). This technique takes advantage of the fact that metabolic byproducts of muscle activity create changes in intracellular water concentration. These changes are measured with mfMRI as changes in transverse relaxation time (T2 signal recorded in ms), which increases as a result of muscle activity.

In a clinical trail, head and neck cancer patients performing swallowing exercises had less muscle atrophy as determined by muscle thickness measurements and T2 relaxation times using MRI (Carnaby-Mann et al., 2011). However, the specificity of swallowing exercises to particular muscle groups underlying laryngeal elevation has not been determined. The aims of this study are twofold: to use mfMRI to determine the relative involvement of muscles underlying hyolaryngeal elevation during swallowing; and to evaluate the ability of two swallowing exercises to target the posterior sling muscles. Our presuppositions are that: i) after swallowing the anterior and posterior sling muscles will show significant effect size changes in whole muscle T2 signal profiles; and ii) the Mendelsohn maneuver and effortful pitch glide will effectively target the posterior sling muscles as demonstrated by significant effect size changes in T2 whole muscle profile of the palatopharyngeus and stylopharyngeus muscles.

Methods

Eleven young, healthy subjects including six males and five females age 22-30 (mean age=25), judged to have normal swallowing ability by a speech-language pathologist were included in this study under a research protocol approved by the Boston University Medical Campus Institutional Review Board. Subjects were trained under biofeedback by a swallowing specialist to perform the Mendelsohn maneuver and the effortful pitch glide swallowing exercises during a Fiberoptic Endoscopic Examination of Swallowing (FEES). Within two weeks of the FEES procedure, mfMRI studies were conducted.

Four separate mfMRI sequences were acquired in each subject: prior to swallowing, after

eight repeated swallows, following eight consecutive Mendelsohn maneuvers, and following eight consecutive effortful pitch glides. Multiple contiguous 4 mm axial images from the cricoid to the hard palate were acquired without intravenous contrast using a 3 Tesla Philips Achieva MRI scanner with 16-channel neurovascular coil. Images were acquired using spin-echo sequences with a repetition time (TR) of 2500ms and dual echo times (TE) of 17.8 and 80ms. Structural T1 weighted scans were acquired in three planes for use as an anatomical cross-reference. Axial 1 mm images were acquired using a fast-field echo sequence with TE/TR of 2.3/15 ms. Sagittal and coronal 3.3 mm images with multiple slice turbo spin echo sequences with TE/TR of 9.2/453.8 ms were also acquired. Two-planar (coronal and sagittal) dynamic MRI scans (T1 weighted fast gradient echo sequence with TE/TR = 0.9/2.4 ms, 10 mm slice thickness, temporal resolution of 8.3fps) were acquired to confirm adequate performance of swallowing exercises.

Timed visual cues administered via PowerPoint™ slides projected to a mirror inside the magnet instructed the subjects to rest, swallow, or perform a specific swallowing exercise. The bolus for repetitive swallowing tasks was 400ml of thin liquid administered via tubing from which the subject drew a self-selected volume of liquid. On all eleven subjects, scans were acquired before and after repeated swallowing. On nine of eleven subjects, scans were acquired after two sets of swallowing exercises with an interval of 20 minutes rest between tasks. As a positive control, the remaining two subjects performed a prolonged bite (80 sec.) to demonstrate that the change in T2 signal does localize to active muscle (Yamaguchi et al., 2011).

Two independent investigators determined mean signal intensities of long and short TE images by segmenting the following muscles: thyrohyoid, mylohyoid, geniohyoid, anterior digastric, posterior digastric/stylohyoid complex, palatopharyngeus, stylopharyngeus, and sternocleidomastoid (control). The masseter was segmented in two subjects as a positive control. Both investigators independently segmented all muscles slice-by-slice using the semi-automated process detailed below.

An automated segmentation algorithm of Osirix digital imaging and communication in medicine (DICOM) software was used to segment muscles (http://www.osirix-viewer.com). The process began by manually selecting a single seed point within the muscle body of interest on each slice. Next, the algorithm automatically calculated the mean and standard deviation of signal intensities of within one voxel surrounding the seed point, and automatically selected *adjacent* voxels that fell within the interval of a standard deviation level set by the investigator (Fig. 23). Additional seed points were then manually selected and a growing region of interest was generated. The investigator accepted or rejected each automated selection based on its visual agreement with the anatomical boundaries of each muscle. This selection process produced a region of interest representing the mean signal intensity of a muscle at a particular slice level. Once a region of interest was determined on one TE slice it was copied and imported to the second, identical TE slice located on the same slice level (to be clear, there are two images per slice level). The process was repeated on all slice levels in which a muscle was identified.

80	73	21	24	23	22	19	18	13
72	62	22	27	21	18	17	9	4
73	57	33	26	28	11	12	8	3
88	98	92	66	21	18	21	11	14
86	97	89	31	**42**	54	37	19	12
91	96	83	89	61	53	42	23	20
78	85	81	89	68	73	73	38	22
79	82	78	92	89	77	74	45	23
21	23	12	17	18	22	23	21	24

Fig. 23. Method for semi-automated muscle segmentation. The Osirix (DICOM) software (http://www.osirix-viewer.com) "growing region of interest" interface uses dynamic thresh-holding by averaging the 8 voxels immediately surrounding a seed point (red box) selected by the investigator (in bold). The algorithm determines the mean and standard deviation of signal intensities of these nine voxels, and then selects all neighboring voxels falling within a standard deviation of the mean set by the investigator (in blue). In this example the mean and standard deviation of the seed point and 8 surrounding voxels is=48.33± 22.88. With the segmentation set at 1SD of the mean, the algorithm will automatically select neighboring voxels within the interval of 25 and 71. In principle, tissue types that match the signal intensity of the seed point are segmented while other voxels are excluded.

An anatomist and radiology resident developed a protocol to identify muscles of interest in the axial plane with the aid of anatomical scans in coronal and sagittal dimensions. Superiorly, the thyrohyoid was identified as the deeper of the infrahyoid muscles distal to the hyoid bone; inferiorly the muscle was identified immediately anterior to the thyroid cartilage. The geniohyoid was carefully differentiated in a position just inferior to the genioglossus muscle between the hyoid and genial tubercles. Only the posterior body of the mylohyoid was measured, as it was deemed more structurally relevant to hyolaryngeal elevation and more reliably identifiable lateral to the sublingual gland and medial to the mandible. The anterior belly of the digastric was readily found inferior and lateral to all muscles in the submental region. Segmentation of the complex created by the posterior belly of the digastric and stylohyoid began within the digastric fossa of the temporal bone; inferiorly, medial to the parotid gland, the posterior digastric is joined by the stylohyoid and continues until just medial to the posterior portion of the submandibular gland. Inferiorly, the longitudinal pharyngeal muscles blend indistinguishably into the pharyngeal constrictor muscles; as a result, only the proximal portion of these muscles were segmented. The palatopharyngeus lies behind the palatine tonsil, between it and the nasopharyngeal mucosa. Segmentation began where the muscle blends into the soft palate, adjacent to the uvula and continued posterolaterally towards the pharyngeal wall until just prior to merging into the pharyngeal constrictor muscles. Stylopharyngeus segmentation began on the medial side of the styloid process, continuing inferiorly until indistinguishable from the pharyngeal constrictor muscles. The sternocleidomastoid and masseter were segmented as control muscles. All muscles were segmented on the right side only.

Both investigators segmented all images with the Radiologist blinded to the test condition of each image series. Segmentations were reconciled between investigators with agreement established by slice number and location determined by coordinate data and visual inspection. Since muscles change shape from slice to slice and subject-to-subject,

signal intensities were weighted according to the number of voxels selected by the algorithm at each slice level and averaged to determine mean weighted signal intensities for the whole muscle. These mean-weighted signal intensities for the whole muscle at long and short TE times were used to calculate a whole muscle T2 signal profile using the following formula:

$$T^2 = \frac{TE_{long} - TE_{short}}{Ln\left(\frac{SI_{short}}{SI_{long}}\right)},$$

where TE= the echo time and SI= the mean signal intensity (Yamaguchi et al., 2011). Inter-rater reliability was determined by Intraclass correlation coefficients of T2 signal profiles for each muscle ranging from ICC=0.76-0.94 (Hopkins, 2000) (Table 3). Results were determined using the mean T2 signal profile from both raters.

Table 3: Inter-rater reliability was calculated using Intraclass Correlation Coefficients of T2 signal profiles

	ICC	Lower 95% confidence limit	Upper 95% confidence limit	F-statistic
Thyrohyoid	0.88	0.79	0.93	15.70
Mylohyoid	0.79	0.64	0.88	8.60
Geniohyoid	0.79	0.64	0.88	5.30
Anterior Digastric	0.92	0.85	0.95	22.60
Post Digastric/Stylohyoid	0.91	0.84	0.95	21.30
Palatopharyngeus	0.76	0.59	0.87	7.40
Stylopharyngeus	0.94	0.88	0.97	30.60

Results

Mean and confidence intervals of T2 signal profiles for muscles under each condition and effect size changes of T2 signal profiles of muscles are reported in Table 4. Effect size changes were used to measure relative activation of muscles post-swallowing (Fig. 24) and post-exercise conditions with significance set at Cohen's $d>.20$ (Fig. 25, 26). Post-hoc corrections to effect sizes were made for a sample size less than twenty (Nakagawa and Cuthill, 2007). Hypothesis testing using a one-tailed paired t-test of anterior and posterior sling muscles demonstrated a statistically significant difference between pre- and post-swallowing conditions ($n=11$) for the geniohyoid ($p=0.01$) and palatopharyngeus ($p=0.04$). Hypothesis testing using a one-tailed t-test for the two swallowing exercises ($n=9$) demonstrated a statistically significant difference between pre-swallowing and post-effortful pitch glide (EPG) in targeting the palatopharyngeus ($p=0.02$), stylopharyngeus ($p=0.05$), and mylohyoid ($p=0.05$). No statistically significant difference were found in the Mendelsohn maneuver (MM).

Table 4: Means and 95% confidence intervals (CI) of T2 signal profiles for muscles under each condition

	Pre Swallowing		Post Swallowing		Post Mendelsohn Maneuver		Post Effortful Pitch Glide	
	Mean	95% CI±	Mean	95% CI±	Mean	95% CI±	Mean	95% CI±
Thyrohyoid	46.08	1.53	47.62	2.79	47.58	3.81	46.60	2.48
Mylohyoid	46.13	1.27	47.13	1.59	49.64	1.47	48.03	1.80
Geniohyoid	48.93	0.80	50.94	1.84	50.12	2.02	50.00	2.15
Anterior Digastric	47.93	1.16	48.07	2.51	50.11	2.84	48.87	1.53
Posterior Digastric/Stylohyoid	44.21	1.93	44.92	1.39	44.98	2.24	45.66	2.15
Palatopharyngeus	49.41	1.13	50.76	2.00	51.11	2.53	51.39	1.46
Stylopharyngeus	53.30	4.05	55.32	4.61	57.47	5.75	58.62	4.22

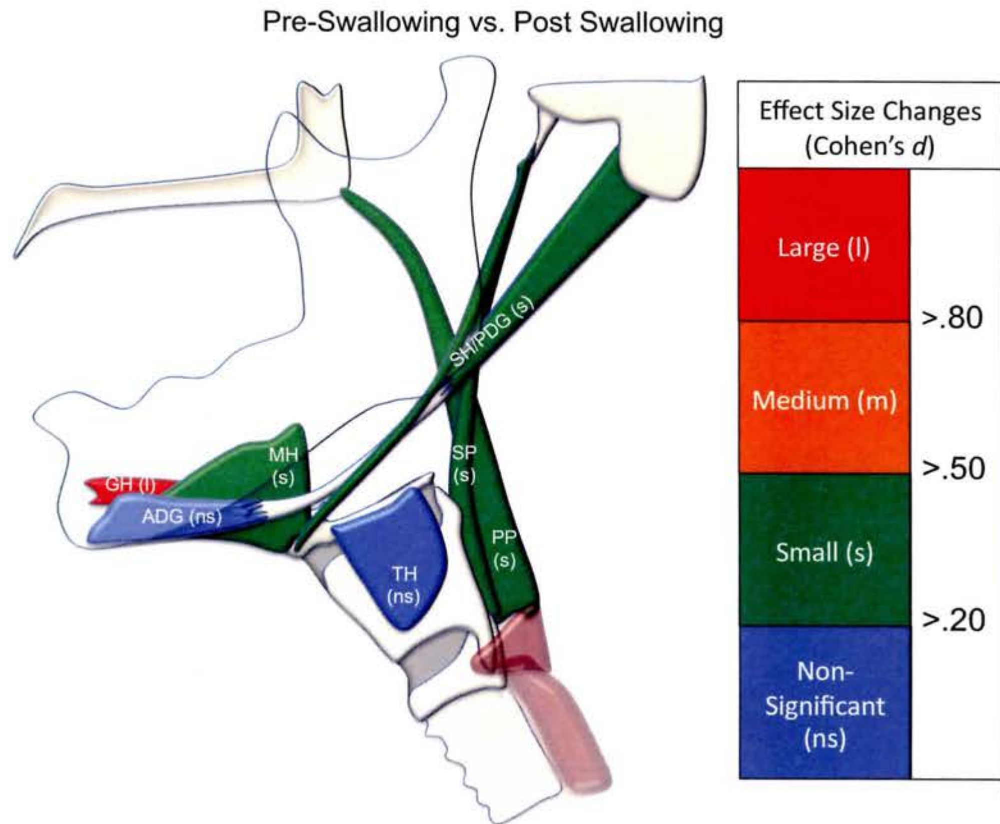

Fig. 24. Pre-swallow vs. post swallow effect size changes of whole muscle T2 signal profiles depicted for each muscle (GH=geniohyoid, ADG=anterior digastric, MH=mylohyoid, TH=thyrohyoid, SH/PDG=stylohyoid/posterior digastric, SP=stylopharyngeus, PP= palatopharyngeus). Cohen's *d* greater than 0.20 is considered significant with >.50 interpreted as a medium effect size and >.80 as a large effect size change. All muscles excepting the thyrohyoid and anterior digastric achieved significant effect size changes.

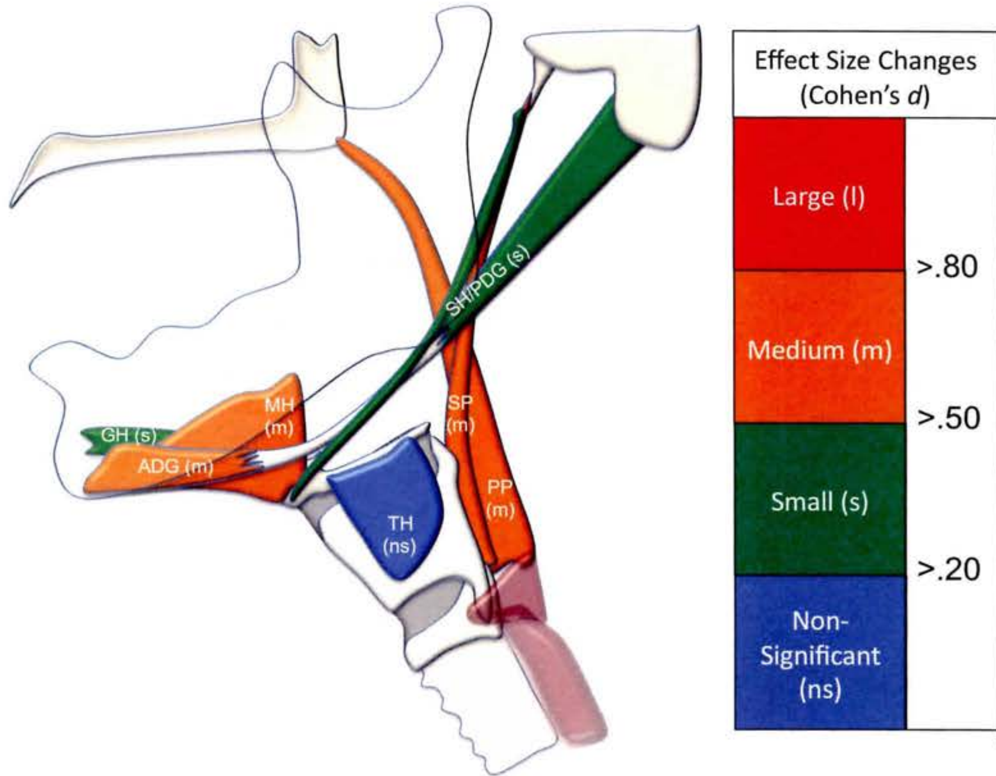

Fig. 25. Pre-swallow vs. post Mendelsohn maneuver effect size changes of whole muscle T2 signal profiles depicted for each muscle (GH=geniohyoid, ADG=anterior digastric, MH=mylohyoid, TH=thyrohyoid, SH/PDG=stylohyoid/posterior digastric, SP=stylopharyngeus, PP= palatopharyngeus). Cohen's *d* greater than 0.20 is considered significant with >.50 interpreted as a medium effect size and >.80 as a large effect size change. All muscles excepting the thyrohyoid achieved significant effect size changes.

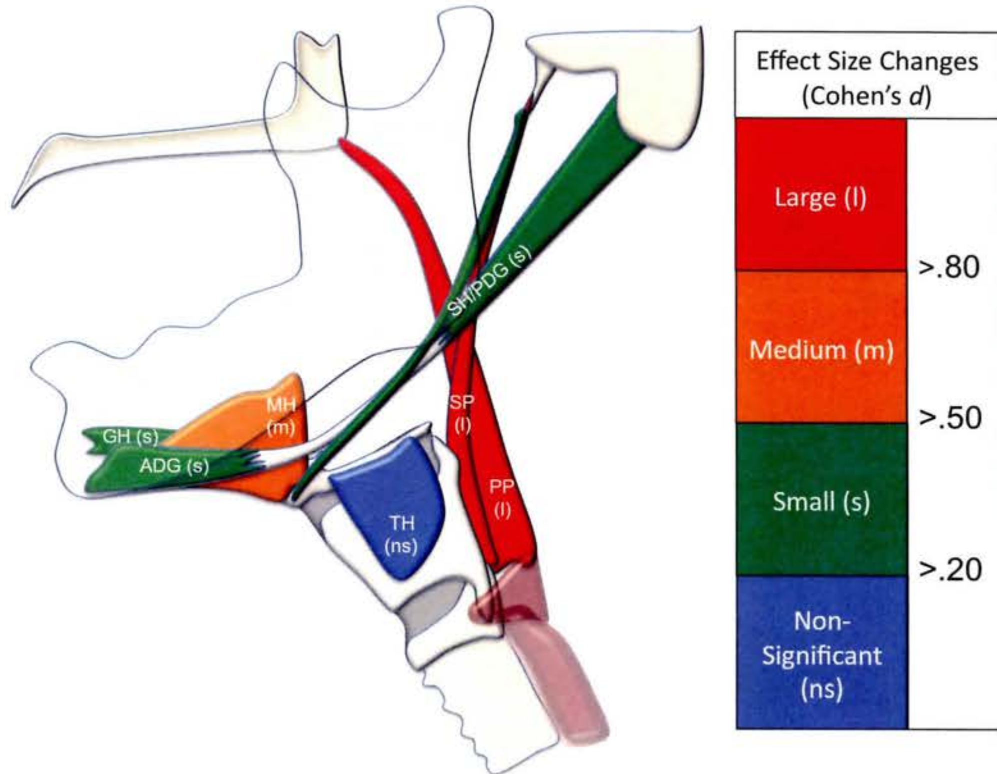

Fig. 26. Pre-swallow vs. post effortful pitch glide effect size changes of whole muscle T2 signal profiles depicted for each muscle (GH=geniohyoid, ADG=anterior digastric, MH=mylohyoid, TH=thyrohyoid, SH/PDG=stylohyoid/posterior digastric, SP=stylopharyngeus, PP= palatopharyngeus). Cohen's *d* greater than 0.20 is considered significant with >.50 interpreted as a medium effect size and >.80 as a large effect size change. All muscles excepting the thyrohyoid achieved significant effect size changes.

The negative control muscle (sternocleidomastoid) showed no increase in T2 values in pre- vs. post-swallowing conditions (Fig. 27a). The positive control muscle (masseter) demonstrated no significant change with swallowing activity but did show significantly higher T2 values after the 80sec bite maneuver using a two-tailed paired *t*-test (p=0.02, n=2) (Fig. 27b).

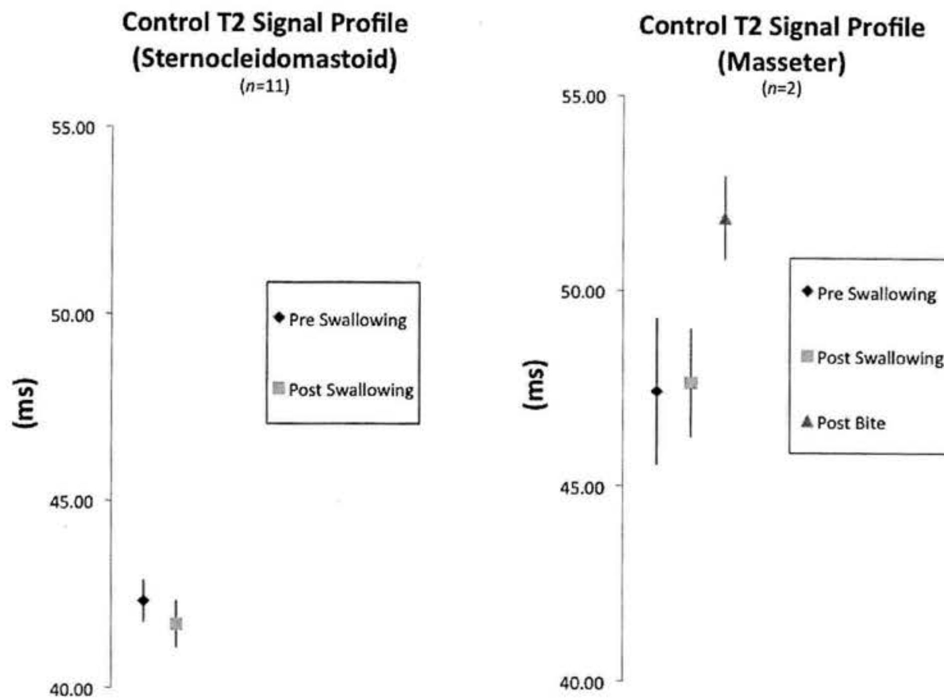

Fig. 27a-b. T2 signal profiles of negative and positive control muscles. (a) Comparison of T2 profiles of negative control muscle (sternocleidomastoid) pre- to post-swallowing. (b) Comparison of T2 signal profiles of masseter before and after swallowing and after a prolonged bite down (80 sec.) in two subjects. Post bite down T2 signal profiles show a significant difference (p=0.02) compared to post swallowing profiles.

Discussion

Reduced elevation of the larynx is a primary predictor of aspiration and is often seen in radiation-induced dysphagia. This study shows that two muscular slings are active in hyolaryngeal elevation, and that the Mendelsohn maneuver and effortful pitch glide effectively target the longitudinal pharyngeal muscles as well as the suprahyoid muscles

as seen by significant effect size changes in whole muscle T2 signal profiles (Fig. 24). Effect size changes in the thyrohyoid did not reach significance. These experimental findings share areas of agreement and disagreement with IMRT studies.

Agreement between our findings and IMRT studies is in identifying the longitudinal pharyngeal muscles as important to deglutition and dysphagia. As noted elsewhere (Christianen et al., 2011; Eisbruch et al., 2004), IMRT demarcation of the "pharyngeal constrictor muscles" is a conflation of muscle groups. The pharyngeal wall incorporates a double layer of muscles including an outer layer of predominately fast-twitch fibers, and an inner layer of predominately slow-twitch fibers (Mu and Sanders, 2001). Therefore, we suggest a more anatomically informative name for this dosage boundary is "pharyngeal wall muscles" versus "pharyngeal constrictor muscles". The outer layer is comprised of the superior, middle and inferior pharyngeal constrictor muscles. The inner layer is comprised of the blended distal fibers of the palatopharyngeus, salpingopharyngeus, and stylopharyngeus, which insert into the thyroid cartilage and pharynx (Pearson et al., 2012). IMRT demarcations for the "pharyngeal constrictor muscles" encompass these distal fibers, but exclude the stylopharyngeus between the styloid process and the pharyngeal wall, and the palatopharyngeus inserting into the palatine aponeurosis.

The IMRT guidelines also make no provision for the thyrohyoid muscle that has long been associated with swallowing, and thought to underlie the approximation of the thyroid to the hyoid. The thyrohyoid has been shown to be active in several electromyography studies of deglutition in animals. Four of the eleven subjects in this study showed a loss in T2 signal lowering the effect size change, while others showed a gain. The variability in the function of the thyrohyoid may indicate that this muscle is not as essential as previously thought.

Disagreement between this study and IMRT findings lies with the importance of the anterior sling (suprahyoid) muscles, which are excluded in current guidelines. The criterion for inclusion in IMRT guidelines was an increase in soft tissue thickness as measured on CT at 3 months post-radiation (Eisbruch et al., 2004). Eisbruch and colleagues discussed that soft tissue thickness may be attributable to inflammation of the mucosa rather than muscles, tissues that are indistinguishable in CT. In other reports, larger soft tissue thickness in response to exercise is assumed to represent increased muscle function (Carnaby-Mann et al., 2011). We argue that measuring the impact of radiation on muscle function solely on the criterion of soft tissue thickness with CT is insufficient to determine if these muscles should be excluded from IMRT guidelines.

It may be that there is wide margin for error in the swallowing mechanism such that one intact muscular sling is sufficient for the task. Such compensation is seen with lateral medullary infarction (Wallenberg's syndrome) that impairs the function of posterior sling muscles. A prolonged activation of submental muscles has been documented in this syndrome, suggesting that the anterior sling musculature may be compensating for the acute loss of posterior sling function (Aydogdu et al., 2001). It is possible that sparing the long pharyngeal muscles with IMRT may prove sufficient to preserve hyolaryngeal elevation and prevent severe dysphagia.

The current study does not address all swallowing events, but is focused on hyolaryngeal elevation using quantitative measurements of swallowing anatomy and physiology. If the primary incentive for sparing swallowing structures with IMRT is to preserve swallowing function, then future studies should utilize variables representing structure-to-function relationships. For example, choosing kinematic outcome variables that represent the function of the longitudinal pharyngeal muscles (posterior thyroid to cranial base approximation) and the pharyngeal constrictor muscles (pharyngeal narrowing) would provide convincing evidence that the IMRT contour of "pharyngeal constrictor muscles" spares the swallowing function of the pharyngeal wall muscles.

An additional contribution of this paper is a methodology using mfMRI to test swallowing muscle function. This method could be applied to test the effects of IMRT on specific muscle function in future studies. In this study we also used this method to establish the salience of various exercise therapies targeting functional groups of muscles. It has been previously reported that the Mendelsohn maneuver targets the submental muscles using electromyography (Wheeler-Hegland et al., 2008). We demonstrated that the Mendelsohn maneuver and effortful pitch glide effectively targeted the longitudinal pharyngeal muscles as well as muscles of the anterior sling. We did not control for equivalent expenditures of muscle effort in various test conditions. It may be that adding repetitions to exercise regimens would modify the magnitude of our results.

Limitations of the study include the fact that subjects were supine when swallowing. This may have had an effect on how muscles were recruited while swallowing in this position. There was also the difficulty establishing a baseline T2 measurement in muscles that are in constant use, as are those of the pharynx. For this reason, we did not provide a prolonged rest period before the pre-swallowing acquisition. Consequently, effect size changes reported here could be underestimated; however, these results more likely represent muscle function in non-experimental settings where patients also do not rest for prescribed periods of time before swallowing.

In conclusion, we document a previously unreported research methodology (whole muscle T2 signal profile) for testing swallowing muscle function. We found that anterior and posterior muscular slings function in swallowing. We also found that while the Mendelsohn maneuver and effortful pitch glide effect anterior sling muscles, both exercises may be utilized to strengthen the posterior sling muscles. While IMRT guidelines currently protect many structures critical to swallowing including the cricopharyngeus muscle, pharyngeal constrictor muscles, longitudinal pharyngeal muscles, and salivary glands, this study shows that guidelines exclude structures important to swallowing function. Whether this exclusion is clinically relevant is

unknown. We suggest that studies designed to investigate structure-to-function relationships in swallowing may result in IMRT guidelines that more fully address radiotherapy-related dysphagia and aspiration.

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Chapter 5

Kinematic Analysis of the Two-Sling Mechanism as Visualized in Dynamic MRI

Abstract

Introduction: Accurately characterizing the movement of the hyolaryngeal complex is important to the field of dysphagia research. In this paper a new method for obtaining kinematic measurements of hyolaryngeal movement using coordinates of anatomical landmarks is demonstrated. This study quantifies the actions during swallowing of the anterior and posterior sling muscles that suspend the hyoid and larynx. These muscles have been shown to be active in swallowing using muscle functional MRI (Chapter 4). Kinematic measurements taken from dynamic MRI images (acquired from subjects in Chapter 4) are used to describe the actions of these muscles. In this study the question of the proper axis of reference for measuring hyoid movement in swallowing is also addressed.

Methods: Two investigators independently mapped nine coordinates, which define three skeletal levers (cranial base, mandible, and vertebrae) and elements of the hyolaryngeal complex (hyoid, laryngeal cartilages and associated structures including the cricopharyngeus forming the upper esophageal sphincter) from video files of dynamic MRI. Coordinates were recorded from two time points: minimum and maximum hyolaryngeal excursion. Coordinates were mathematically converted into multiple kinematic measurements of hyolaryngeal movement. Inter-rater reliability was evaluated by Intraclass correlation coefficients of calculated measurements. Hyoid excursion, the distance of hyoid displacement, was measured from two different axes of reference (mandible and vertebrae) to determine if the axis of reference chosen altered the result when measuring hyoid movement.

Results: All kinematic measurements of hyolaryngeal excursion indicated concentric contraction (active shortening) of the anterior and posterior muscular slings. Hyoid excursion measurements using the vertebrae as an axis of reference and hyoid excursion measurements using the mandible as an axis of reference yielded significantly different results ($p=0.002$).

Discussion: The action of the two-sling mechanism functions to elevate the hyolaryngeal complex in normal swallowing. Obtaining landmark coordinates is a reliable method to generate multiple kinematic variables from lateral view imaging modalities. The mandible is a more reasonable anatomical axis of reference for measuring hyoid movement.

Chapter 5

Kinematic Analysis of the Two-Sling Mechanism as Visualized in Dynamic MRI

Introduction

A primary movement in normal swallowing is hyolaryngeal elevation to help protect the airway and open a relaxed upper esophageal sphincter. Therefore, characterizing the movement of the hyoid and larynx is fundamental to the study of deglutition and dysphagia (Kim and McCullough, 2009; Leonard et al., 2000; Logemann et al., 2000; Perlman et al., 1995; Steele et al., 2011). The hyolaryngeal complex is an interconnected set of structures including the hyoid bone, laryngeal cartilages, and associated muscles and ligaments that incorporate the upper trachea and esophagus (Fig. 28). The hyoid is contiguous with the thyroid cartilage via the thyrohyoid muscle and membrane: the upper esophageal sphincter is formed primarily by the cricopharyngeus contiguous with the cricoid cartilage. A proposed mechanism for hyolaryngeal elevation is by the action of two muscular slings that suspend the hyolaryngeal complex from the mandible and cranial base (Pearson et al., 2012, Chapter 3). Physiological data show that these muscular slings function during swallowing (Van Daele et al., 2005, Chapter 4). The purpose of this study is to determine the actions of this two-sling mechanism.

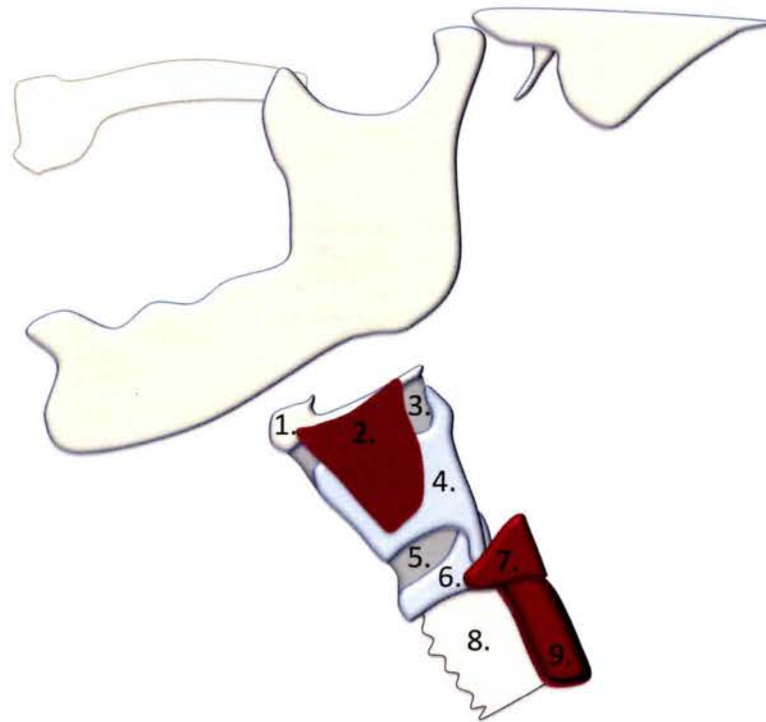


Fig. 28. The hyolaryngeal complex includes the: 1.) hyoid, 2.) thyrohyoid muscle 3.) thyrohyoid membrane, 4.) thyroid cartilage, 5.) cricothyroid membrane, 6.) cricoid cartilage, and 7.) cricopharyngeus. The 8.) trachea and 9.) esophagus are incorporated into the hyolaryngeal complex. Elevation of this complex helps to protect the airway and open a relaxed upper esophageal sphincter.

The two slings proposed to elevate the hyolaryngeal complex are the anterior and posterior muscular slings. The anterior sling is comprised of the suprahyoid muscles (mylohyoid, geniohyoid, stylohyoid, and digastric) assisted by the thyrohyoid, and has long been associated with elevation of the hyoid and larynx during deglutition (Cook et al., 1989; Sivarao and Goyal, 2000). Anatomical studies have shown that the mylohyoid and geniohyoid have the greatest potential for displacing the hyoid bone (Pearson et al., 2011, Chapter 2). Intramuscular electrical stimulation of the mylohyoid, *geniohyoid and*

thyrohyoid has been shown to elevate the larynx (Burnett et al., 2003). Irregularities in hyoid excursion are associated with disordered swallowing (Bingjie et al., 2010; Steele et al., 2011).

The posterior sling consists of the longitudinal pharyngeal muscles (stylopharyngeus, palatopharyngeus and salpingopharyngeus). Injury to these muscles is thought to underlie dysphagia and aspiration, and as such their exposure to radiation is minimized during cancer treatments using tissue-sparing technologies such as intensity-modulated radiotherapy (Christianen et al., 2011; Eisbruch et al., 2004; Feng et al., 2007; Levendag et al., 2007). Anatomical studies show that the longitudinal pharyngeal muscles morphologically have significant potential for elevating the larynx (Meng et al., 2008; Okuda et al., 2008; Pearson et al., 2012c). A swallowing event called pharyngeal shortening likely represents the action of the posterior sling muscles elevating the posterior aspect of the hyolaryngeal complex including the upper esophageal sphincter (Kahrilas et al., 1992).

In chapter 4, muscle functional MRI (mfMRI) was used to determine which of the anterior and posterior sling muscles function during swallowing. Significant effect size changes were found in all muscles of interest except the anterior digastric. Anterior sling muscles indicated by mfMRI as active in swallowing include the mylohyoid, geniohyoid, and the posterior digastric-stylohyoid complex. Previous anatomical studies have shown that morphologically these muscles have the potential to approximate the hyoid anterior-superiorly toward the mandible (Pearson et al., 2011). Posterior sling muscles indicated by mfMRI as active in swallowing include the palatopharyngeus and the stylopharyngeus. The purpose of this study is to determine if the muscles shown to be active using mfMRI elevate the hyolaryngeal complex thereby stretching open the upper esophageal sphincter.

Videofluoroscopy is a clinical assessment imaging modality. It is also used in research to quantify the multiple movements of the hyolaryngeal complex (Leonard et al., 2000). Distance measurements can be acquired using dynamic MRI that are similar to those acquired using videofluoroscopy, but without exposure of the research subjects to radiation (Honda and Hata, 2007). While kinematic measurements are essential to understanding swallowing function, use of different axes of reference and scalars of measurement in different studies results in findings that are often inconsistent among the various methods of kinematic measurements (Molfenter and Steele, 2010). To overcome this limitation we developed a methodology that utilizes coordinates of salient features of the two-sling mechanism (Pearson et al., 2012). Trigonometric conversion of anatomical coordinates can generate multiple kinematic measurements of interest. This method can be applied using either imaging modality, enabling cross-study comparisons.

For example, Molfenter and Steele pointed out that different investigators measure the position of the hyoid against different axes of reference. If one axis of reference produces significantly different results than another, it would be important to know since hyoid excursion has been suggested as a biomarker for risk of aspiration (Steele et al., 2011). Using coordinates, measurements can be calculated against any chosen axis of reference for comparison.

In this method, five coordinates map the position of three skeletal levers (cervical vertebrae, the mandible, and the hard palate contiguous with the cranial base), and four additional coordinates map features of the hyolaryngeal complex in each frame of reference (Fig. 29). During swallowing, each skeletal lever and feature of the hyolaryngeal complex is in motion, as is the patient, and in videofluoroscopy the fluoroscope under manual clinician control also is moving. This makes the accurate measurement of a moving landmark from frame to frame, difficult. By collecting coordinates, the distance of multiple features in reference to various axes can be accurately measured in each image frame.

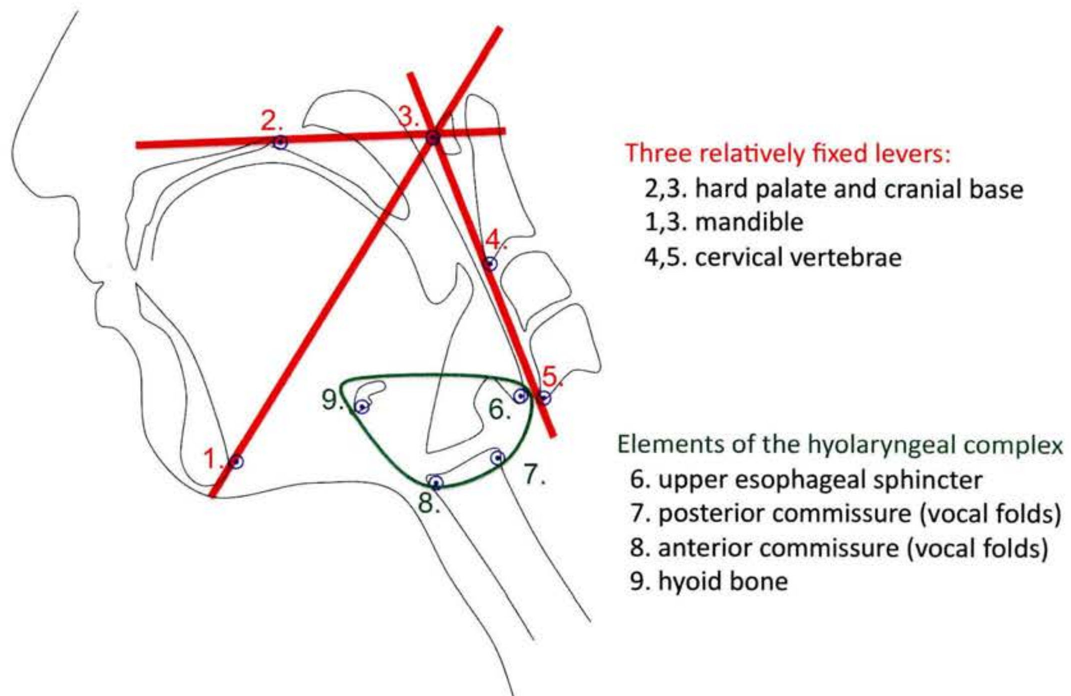


Fig. 29. The anatomical landmarking method uses five coordinates to map three skeletal levers and four coordinates to map elements of the hyolaryngeal complex

The primary aim of this study was to document the action of the anterior and posterior muscular slings. To address this question we utilized a novel anatomical landmarking method to calculate the excursion distances of these landmarks from minimum to maximum swallow. The landmarks approximate the underlying attachments of muscles, so their changing locations during a swallow approximate the actions of these muscles. Importantly, data from Chapter 4 show the muscles to be active, minimizing the likelihood that length changes are merely passive. Our measurements included: hyoid

excursion representing the action of the suprahyoid muscles; hyolaryngeal approximation representing the action of the thyrohyoid (though this action may be assisted by stylopharyngeus); posterior laryngeal elevation to the cranial base representing the action of the stylopharyngeus, and the upper esophageal sphincter approximating toward the posterior edge of the hard palate representing the action of the palatopharyngeus. This action of the palatopharyngeus is here referred to as pharyngeal shortening.

We also used this system of coordinates to address the question raised by Molefenter and Steele concerning the importance of the axis of reference in measuring hyoid movement. In this part of the study we compared measurements of hyoid excursion in reference to the vertebrae with hyoid excursion in reference to the mandible derived from the same set of coordinates.

Methods

Six males and five females age 22-30 (mean age=25) were recruited for this study under a research protocol approved by the Boston University Medical Campus Institutional Review Board. All eleven subjects were judged to have normal swallowing ability by a speech-language pathologist. In a previous study we reported whole muscle T2 signal profiles derived from mfMRI acquired from these subjects (Pearson et al., 2012a). In this same series of acquisitions, two-planar T1 weighted dynamic MRI images were acquired from subjects during a repeated swallow task. Subjects drew the bolus from a tube connected to a container of 400ml of magnesium infused thin liquid. Magnesium is a natural contrast agent in T1 weighted images. Two planar dynamic MRI adds the dimension of time to a two dimensional image acquisition in alternating planes (sagittal and coronal). In this study only the sagittal images were used. Acquisition parameters included: T1 weighted fast gradient echo sequence with TE/TR = 0.9/2.4 ms, 10 mm slice thickness, and a temporal resolution of 4.3 frames per second.

A QuickTime™ video file of a series of sagittal images of swallowing was produced with Osirix digital imaging and communication in medicine (DICOM) (http://www.osirix-

viewer.com). Nine easily identifiable anatomical landmarks which map three skeletal levers and features of the hyolaryngeal complex were selected for this analysis based on prior anatomical research and a method originally developed for analyzing videofluoroscopy (Pearson et al., 2012b)(Fig. 30a-b.). The first five coordinates tracked the relative position of the cervical vertebrae, mandible and cranial base as follows: the anterior-inferior edge of C2 and C4 vertebrae; the mandible at the inferior genial tubercles; the hard palate crossed by the anterior edge of the mandibular ramus; and the tubercle of the atlas representing a two-dimensional pivot for all three levers. The four remaining coordinates mark features of the hyolaryngeal complex as follows: the anterior inferior edge of the hyoid; the anterior and posterior commissures of the vocal folds indicating an anterior and posterior laryngeal coordinate; and the inferior point of the hypopharynx air column proximal to the upper esophageal sphincter. These same coordinates can be marked using the sagittal plane dynamic MRI scans (Fig. 31).

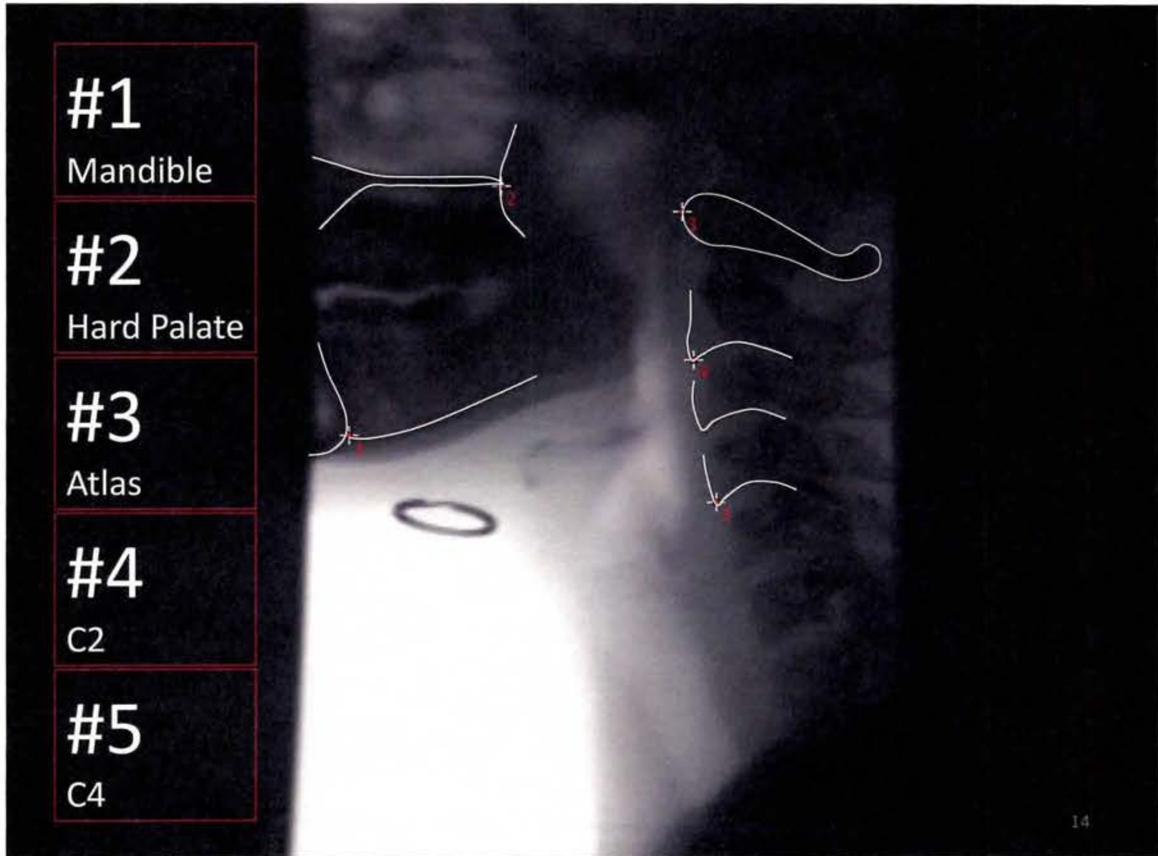


Fig. 30a. Landmarks for five coordinates (1-5) that map three skeletal levers are identified and pictured here using videofluoroscopy.

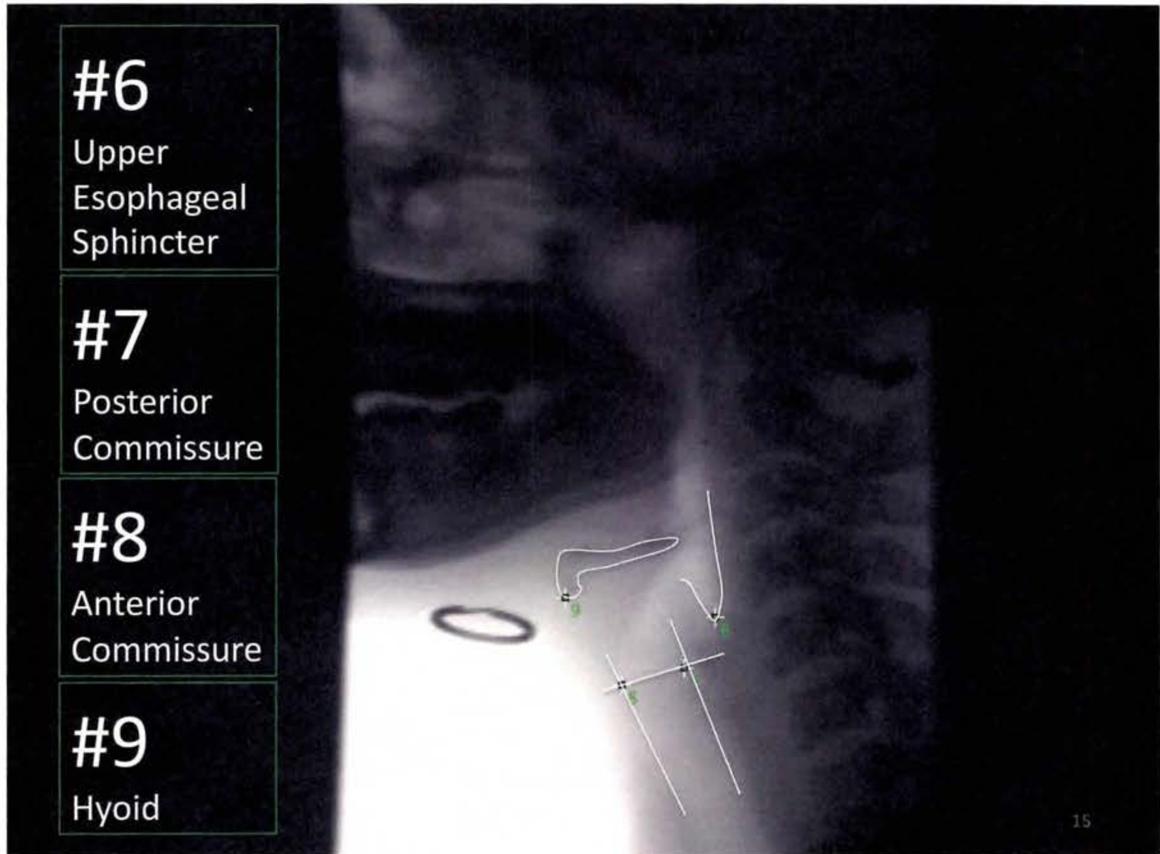


Fig. 30b. Landmarks for four coordinates (6-9) that map the elements of the hyolaryngeal complex are identified and pictured here using videofluoroscopy.

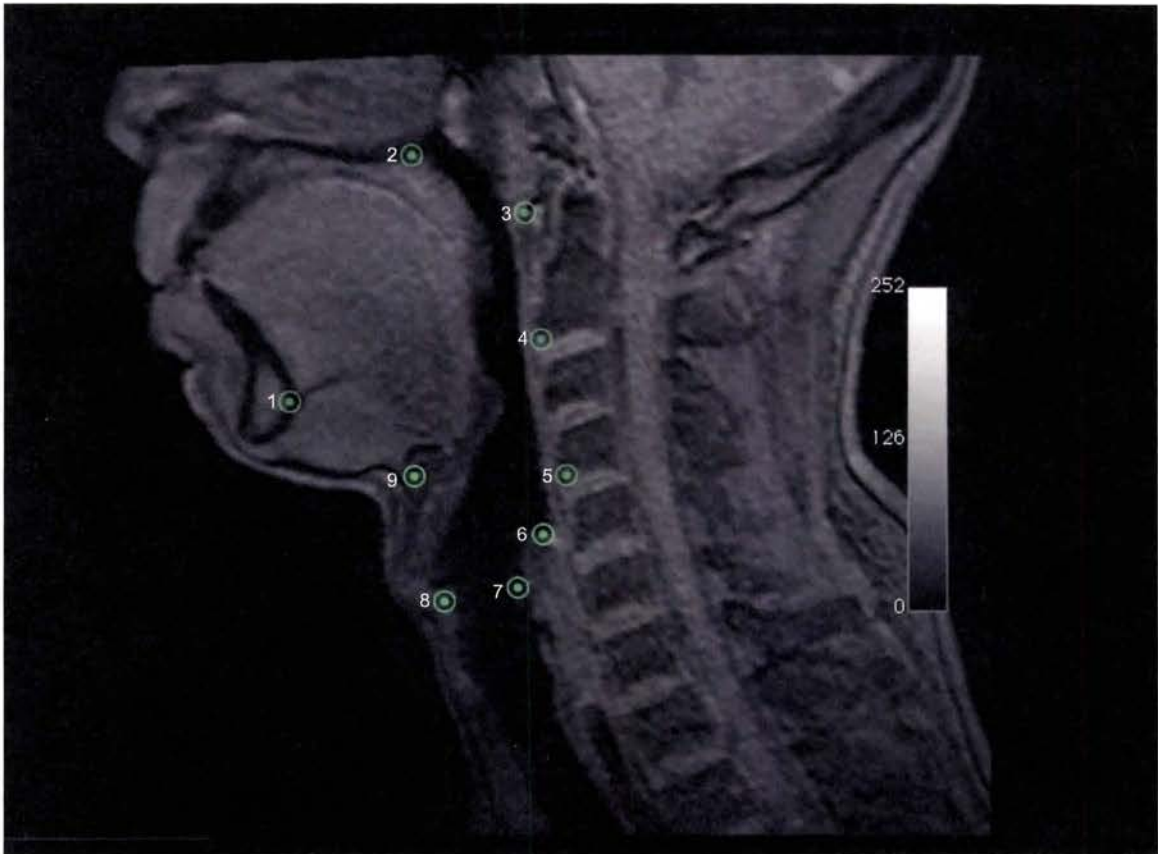
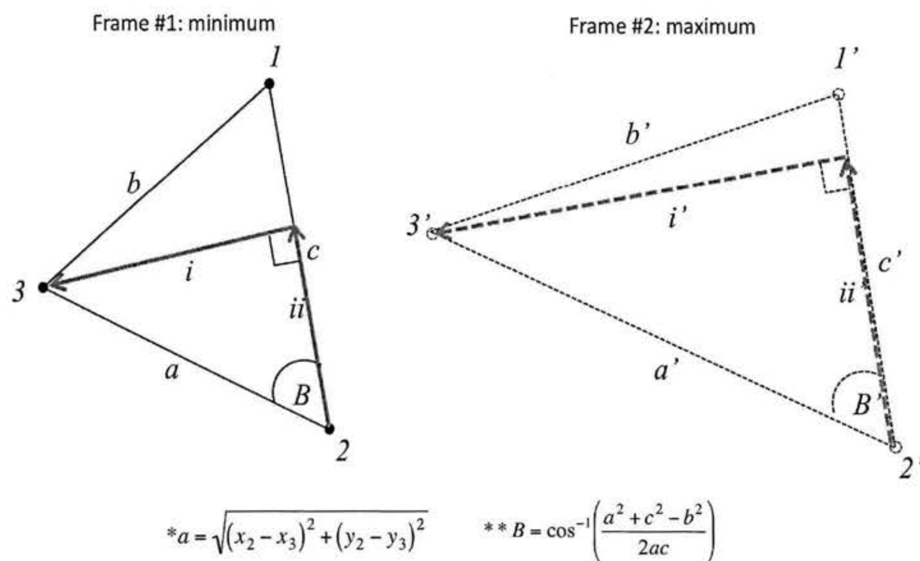


Fig. 31. Nine coordinates mapping the features of the two-sling mechanism for hyolaryngeal elevation is mapped here on a T1 weighted sagittal plane dynamic MRI. 1=genial tubercles of the mandible, 2=posterior edge of the hard palate, 3=anterior tubercle of C1, 4=anterior inferior edge of C2, 5=anterior inferior edge of C4, 6=inferior air column of hypopharynx proximal to the upper esophageal sphincter, 7=posterior commissure of the vocal folds (posterior larynx), 8=anterior commissure of the vocal folds (anterior larynx), 9=anterior inferior edge of the hyoid.

To calculate measurements of hyolaryngeal movement, 20 coordinates were collected from each video file using the multiple-point tool in ImageJ image analysis software equipped with QuickTime™ plug-ins (http://rsbweb.nih.gov/ij). Nine coordinates were recorded from the frame preceding hyolaryngeal elevation relevant to pharyngeal swallowing. Nine more were recorded from the frame at maximum elevation of the hyolaryngeal complex. The two remaining coordinates were recorded from each end of a ten-centimeter scalar recorded on the image by Osirix. Two investigators (an anatomist and a speech-language pathologist) charted all coordinates independently. Coordinates were then imported into a spreadsheet containing trigonometric formulas that converted coordinate data into kinematic measurements of interest (Fig. 32)(See Appendix A for details of all measurements).



$$*a = \sqrt{(x_2 - x_3)^2 + (y_2 - y_3)^2} \qquad **B = \cos^{-1}\left(\frac{a^2 + c^2 - b^2}{2ac}\right)$$

Fig. 32. Trigonometric transformation of coordinate data into the excursion of the hyoid using the axis of the vertebrae. Point 1=*x,y* coordinate of C1, point 2=*x,y* coordinate of C4, and point 3=*x,y* coordinate of the hyoid. *c*=axis of reference, *B*=angle of interest, *a*=hypotenuse. *Any distance between coordinates is derived using Pythagoras' theorem as demonstrated by length *a*. ** Any angle of interest is derived using the law of cosines as demonstrated by angle *B* by using calculations of lines *a,b,* and *c*. Anterior displacement of point *3* in reference to the axis of reference (line *c*) is =*i'-i*, where *i'=sin(B')a'* and *i=sin(B)a*. Superior displacement of point *3* in reference to the axis of reference (line *c*) is = *ii'-ii*, where *ii'=cos(B')a'* and *ii=cos(B)a*. Total excursion distance is calculated by applying Pythagoras' theorem to the total anterior and superior displacement of the hyoid with the hypotenuse equaling the total excursion distance. These formulas, here applied to hyoid excursion in reference to the vertebrae, can be utilized to map the movement of any feature against a chosen axis of reference in any frame of measurement.

Five measurements characterizing hyolaryngeal kinematics were calculated from the same set of coordinate data including: hyolaryngeal approximation (Leonard et al., 2000); hyoid excursion in reference to the vertebrae (Leonard et al., 2000); hyoid excursion in reference to the mandible (approximating the suprahyoid muscles), pharyngeal shortening approximating the attachments of the palatopharyngeus muscle; posterior laryngeal excursion (toward the cranial base) approximating the attachments of the stylopharyngeus (Pearson et al., 2011; Pearson et al., 2012c) (Fig. 33). Please see appendix for detailed calculations of kinematic measurements. All measurements are reported in centimeters, and as a ratio of C2-4 length to adjust for differences in body size (Steele et al., 2011). C2-4 was chosen, as it is may account for the size of the pharynx more accurately than subject height. In our sample C2-4 length and subject height were highly correlated with a Pearson product-moment correlation coefficient of r=0.92. Intraclass correlation coefficients (ICC) were used to evaluate inter-rater reliability of these calculations (Table 5).

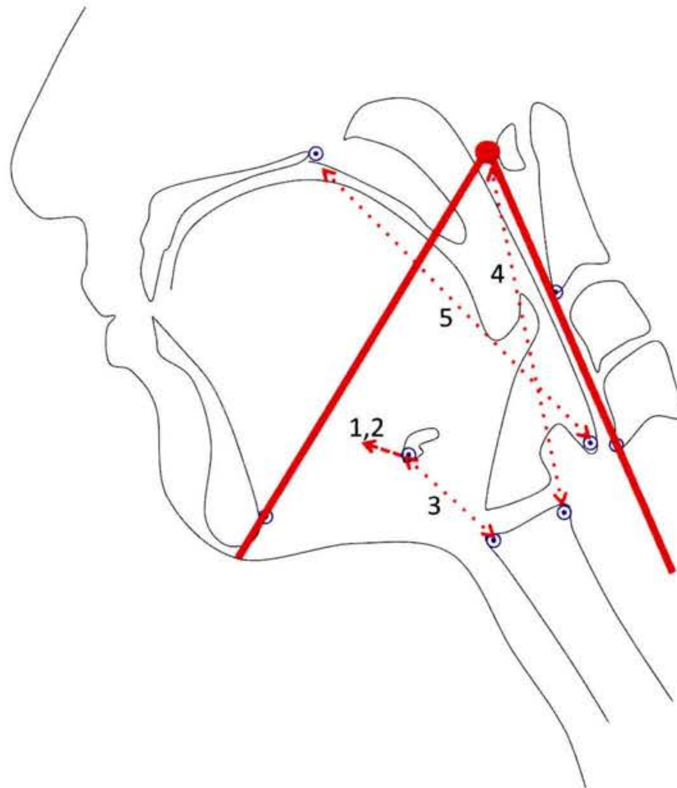


Fig. 33. Calculated distance measurements of: (1) Hyoid excursion with vertebrae as a reference (Anterior Muscular Sling); (2) Hyoid excursion with mandible as a reference (Anterior Muscular Sling); (3) hyolaryngeal approximation (thyrohyoid); (4) Posterior laryngeal elevation to cranial base (Posterior Muscular Sling); and (5) Pharyngeal shortening (Posterior Muscular Sling). Each measurement was reported in centimeters and against a subject specific anatomical scalar of C2-4 length.

Table 5. Inter-rater reliability determined by Intraclass Correlation Coefficients of all kinematic variables calculated from coordinates collected by two independent investigators

	Hyoid Excursion (Mandible)	Hyoid Excursion (Vertebrae)	Hyolaryngeal Approximation	Posterior laryngeal elevation towards the cranial base	Pharyngeal Shortening
Intraclass correlation coefficient	0.94	0.91	0.91	0.82	0.91
Lower 95% confidence limit	0.80	0.70	0.69	0.45	0.72
Upper 95% confidence limit	0.98	0.98	0.97	0.95	0.98

Measurements calculated from coordinate data obtained by the principal investigator were used to compare results of hyoid excursion calculated with the vertebrae as axis of reference versus hyoid excursion with the mandible as axis of reference with a paired two-tailed *t*-test.

Results

Means and standard deviations of all measurements representing the anterior muscular sling (hyoid excursion) and posterior muscular sling (posterior laryngeal elevation to the cranial base and pharyngeal shortening) indicate these muscles shorten to elevate the hyolaryngeal complex (Fig. 34)(Table 6). Hyolaryngeal approximation was inconsistent. In 4 of 11 subjects the distance between the hyoid and larynx increased between minimum and maximum hyolaryngeal excursion, which is contrary to the predicted decrease in distance. A two-tailed paired *t*-test showed that hyoid excursion distances calculated in reference to the vertebrae and the mandible differed significantly (p=0.002) with the mean hyoid excursion measured from the vertebrae reporting 0.81 cm greater than when measured against the mandible (see Table 2).

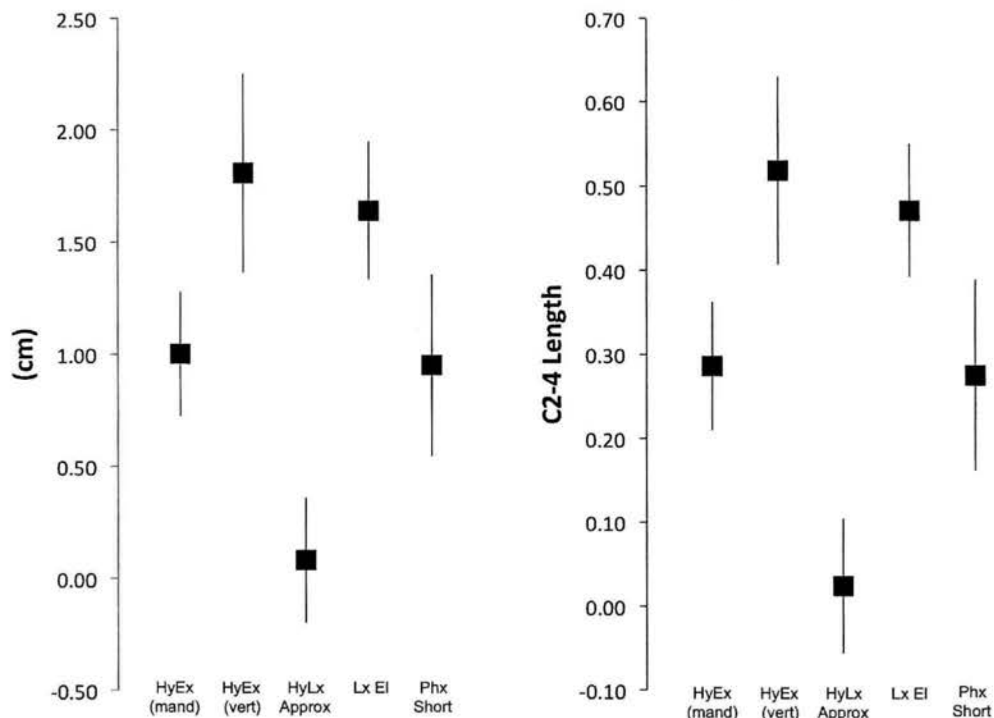


Fig. 34. Means and 95% confidence intervals of kinematic measurements calculated from one set of coordinates per subject (*n*=11) are illustrated. The graph on the left is scaled by centimeters whereas the graph on the right is a ratio of the indicated measurement to the C2-4 length to adjust for between subject size differences. HyEx (mand)= hyoid excursion measured against the axis of the mandible, HyEx (vert)=hyoid excursion measured against the axis of the vertebrae, HyLx Approx= hyolaryngeal approximation, LxEl= Posterior laryngeal elevation to the cranial base, Phx short= pharyngeal shortening representing the action of the palatopharyngeus.

Table 6. Means and standard deviations of kinematic measurements calculated as centimeters and scaled to C2-4 lengths indicate that the action of the two-sling mechanism is to elevate the hyolaryngeal complex.

	Hyoid Excursion (Mandible) (cm)	Hyoid Excursion (Vertebrae) (cm)	Hyolaryngeal Approximation (cm)	Posterior laryngeal elevation towards the cranial base (cm)	Pharyngeal Shortening (cm)
Mean (cm)	1.00	1.81	0.08	1.64	0.95
SD (cm)	0.47	0.75	0.47	0.52	0.68
Mean (C2-4 length)	1.00	1.81	0.08	1.64	0.95
SD (C2-4 length)	0.47	0.75	0.47	0.52	0.68

Discussion

In this paper we found that anterior and posterior sling muscles act to elevate features of the hyolaryngeal complex in swallowing in young healthy subjects. Hyoid excursion towards the mandible represents the summed action of the suprahyoid muscles. Posterior laryngeal elevation to the cranial base represents the action of the stylopharyngeus. Pharyngeal shortening, measuring the approximation of the landmark representing the upper esophageal sphincter to the posterior edge of the hard palate, represents the actions of the palatopharyngeus. We also demonstrated a useful and reliable new method utilizing coordinate data of anatomical landmarks to calculate multiple kinematic measurements of hyolaryngeal movement in swallowing. We found that using different axes of reference for measuring the excursion of the hyoid bone produced significantly different results.

The finding that hyolaryngeal complex is elevated and the pharynx is shortened in swallowing is not new. What is new is mapping these movements to underlying musculature in subjects where specific muscles have been verified as active using mfMRI. Taken together, these data strongly support the idea that muscles of the two-sling mechanism elevate features of the hyolaryngeal complex in swallowing.

An unexpected finding was the inconsistency of hyolaryngeal approximation in different subjects. Consistent approximation of the larynx to hyoid has been reported in a bolus controlled videofluoroscopic study of normal adults with a larger sample size (Leonard et al., 2000). However, the current finding is consistent with the muscle functional MRI findings reported in the last chapter. In chapter 4 it was found that there was no significant increase in mean transverse relaxation times in the thyrohyoid muscle indicating the thyrohyoid was not consistently put to use. The concentric contraction of the thyrohyoid is thought to underlie hyolaryngeal approximation. However, anatomical studies show that portions of the posterior sling muscles insert on the thyroid cartilage and may also cause this same movement. A limitation of the present study is the low temporal resolution of dynamic MRI. While unlikely, it is possible that the imaging study missed the completed approximation of the hyoid and larynx.

What is consistent in both studies is that muscle functional and kinematic measurements are highly variable. Large standard deviations of kinematic measurements indicate the extent of these movements varies extensively between subjects. It is possible that uncontrolled bolus size could also account for this variance (Leonard et al., 2000). It may also be that muscles are recruited differently from subject to subject.

Kinematic measurements are important to our understanding of particular swallowing events. Using a system of coordinates allows for multiple calculations of kinematic measurements that can be tailored to address specific research questions. Using coordinates to calculate measurements reduces time and measurement error introduced by hand-measuring the distance between landmarks. This methodology could also be applied to a number of other imaging modalities where coordinates can be obtained, such as ultrasound or 320-detector-row Multi-slice CT (Inamoto et al., 2011). In the future, this methodology could be the underlying paradigm for a semi-automated software program that provides clinicians with kinematic measurements at the time of a videofluoroscopic imaging study.

Hyoid excursion in reference to the mandible is a novel kinematic measurement designed to more accurately reflect the underlying functional anatomy compared to current hyoid measurements (Pearson et al., 2011). Two studies associating hyoid movement and aspiration found differing results; one found that reduced superior movement of the hyoid was associated with aspiration while the other found that reduced anterior movement of the hyoid was associated with aspiration (Bingjie et al., 2010; Steele et al., 2011). Both measured hyoid movement in reference to the vertebrae. From an anatomical perspective, to better determine if hyoid movement is a biomarker of aspiration, hyoid movement should be measured in relationship to the skeletal levers to which muscles displacing the hyoid attach, rather than the vertebra to which they do not attach.

In sum, we used a novel method using coordinates to calculate kinematic measurements. Using this method we demonstrated the importance of understanding underlying anatomical structure when developing measurements characterizing swallowing function by investigating different methods for measuring hyoid movement. More important to this thesis, kinematic measurements representing the muscles of the anterior and posterior slings shorten. Remembering that these same muscles were found to be active in Chapter 4 allows for the suggestion that the anterior and posterior muscular slings elevate the hyolaryngeal complex in the pharyngeal phase of swallowing.

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Chapter 6

Evaluating Shape Changes Generated by a Two-Sling Mechanism of Hyolaryngeal Elevation in Swallowing

Abstract

Introduction: While kinematic measurements indicate that anterior and posterior sling muscles elevate elements of the hyolaryngeal complex, it has not been determined whether the two slings function as part of a coordinated system. If the two slings function as a mechanism, the slings and associated skeletal elements must work together, or covary. To make this determination, principal components analysis (PCA), a multivariate analysis of covariance, is applied to coordinates of anatomical landmarks collected from dynamic MRI images at different time points.

Methods: A coordinate system described in chapter 5 is used to map elements of the proposed two-sling mechanism of hyolaryngeal elevation. Coordinates are collected from each dynamic (frame) of a dynamic MRI swallowing series of a randomly selected subject from chapter 5 in order to demonstrate shape changes in a single subject. Coordinates collected in chapter 5, representing minimum and maximum hyolaryngeal elevation of all 11 subjects, were also used to demonstrate shape changes of the system among all subjects. MophoJ software was used to perform a Procrustean fit of coordinates, generate a covariance matrix, and perform a principle component analysis of both sets of coordinates. Eigenvalues were used to determine what portion of the total variance is explained by each principal component of covariance. Classifiers were used to determine which principle component is associated with hyolaryngeal elevation. Within this principal component, vectors of shape change (eigenvectors) for each coordinate are reported and illustrated.

Results: For both PCAs hyolaryngeal elevation accounted for the first principle component of variation. For the single subject PCA, the first principle component accounted for 81.5% of the variance. For the between subjects PCA, the first principle component accounted for 58.5% of the variance. Eigenvectors and shape changes associated with this first principle component are reported.

Discussion: Eigenvectors of coordinates associated with hyolaryngeal excursion indicate that two-muscular slings and associated skeletal elements function as components of a covariant system to elevate the hyolaryngeal complex. Morphological analysis is useful to demonstrate changes in the two-sling mechanism of hyolaryngeal elevation.

Chapter 6

Evaluating Shape Changes Generated by a Two-Sling Mechanism of Hyolaryngeal Elevation in Swallowing

Introduction

Characterizing the movement of the hyoid and larynx is important to understanding ordered and disordered swallowing. In the last chapter, we used kinematic measurements representing the effect of the action of the anterior sling muscles (suprahyoid muscles) and posterior sling muscles (long pharyngeal muscles) on various elements of the hyolaryngeal complex. Kinematic measurements are useful in describing particular elements of swallowing physiology, but do not describe these elements in the context of a dynamic system. We have yet to determine if these two muscular slings are components of a coordinated mechanism that elevates the hyolaryngeal complex in swallowing. It is possible, for example, that hyoid elevation alone (attributed to the action of the suprahyoid muscles) could account for shortening the distance between the posterior commissure of the vocal folds (marking the posterior larynx) and the cranial base rather than the active shortening of stylopharyngeus. Using coordinates of anatomical landmarks that map features of the entire dynamic system over time, covariant shape changes can be analyzed by multivariate morphological analysis to determine if these two muscular slings are components of a unified mechanism underlying hyolaryngeal elevation within the context of the swallowing system (Webster et al., 2010).

To characterize the morphology of the swallowing apparatus, we developed a method of tracking changes using nine anatomical landmarks (described in Chapter 5)(Fig. 35). The first five coordinates track the relative position of three skeletal levers including: the vertebrae, mandible and cranial base. The four remaining coordinates mark features of the hyolaryngeal complex including the hyoid, anterior larynx, posterior larynx, and upper esophageal sphincter.

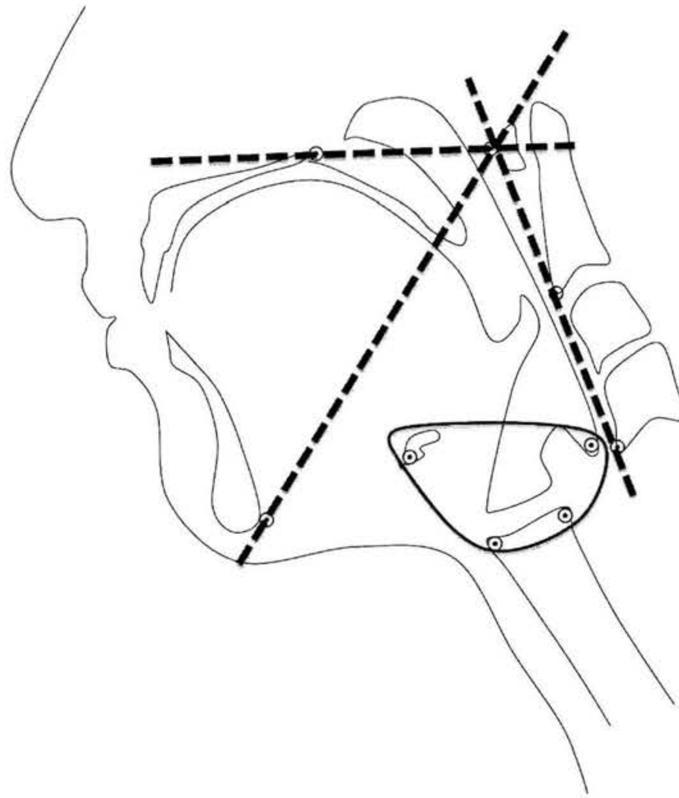

Fig. 35. Nine anatomical landmarks tracking skeletal and muscular elements of a proposed two-sling mechanism for hyolaryngeal elevation. Five coordinates track three skeletal levers illustrated by dashed lines including the vertebrae, mandible and cranial base continuous with the hard palate. The four coordinates, circled with the solid gray line, map interconnected features of the of the hyolaryngeal complex including the hyoid bone, anterior commissure of the vocal folds (anterior larynx), posterior commissure of the vocal folds (posterior larynx), and the inferior hypopharyngeal air column proximal to the upper esophageal sphincter.

In the current study we investigate whether the two muscular slings work together to elevate the hyolaryngeal complex using principal component analysis to analyze shape changes. Principal component analysis is a dimensionless multivariate analysis of a covariance matrix of coordinate data that has been fitted by Procrustean superimposition. The Procrustean fit adjusts for rotation of images and overall size differences. The shape changes associated with movements of landmarks during swallowing can be demonstrated by comparing the covariance of Procrustean coordinates at different time points in the swallow.

Principal component analysis (PCA) is a linear transformation of multiple variables that identifies the sources of covariance between the variables. In situations where there are only two variables, a scatter plot may be used to demonstrate the primary source of covariance (the best-fit line) and the secondary source of covariance (the scatter orthogonal to the best-fit line). PCA functions similarly, but with three or more variables. Each main source, or principal component, of variance within the covariance matrix is determined, such that the first principle component describes the greatest amount of covariance in the matrix, and the second principle component describes the second greatest amount of covariance and so on. These principal components are assigned eigenvalues. Eigenvalues, similar to squared correlation coefficients in bivariate data, describe what portion of the total variance is explained by each principal component. Within each principal component, vectors of shape change (eigenvectors) for each coordinate are determined and used to demonstrate shape changes associated with each principal component. These vectors are not to be interpreted as actual mean distance measurements but rather the magnitude and direction of variance of a particular coordinate within a covariant system. Shape changes are represented by consistent vectors of change for each coordinate in the context of the entire system.

In this study we were interested in the overall action of the anterior and posterior muscular slings during swallowing. By analyzing shape changes of the mechanism using

PCA, the effect of the two-sling mechanism was evaluated. We hypothesized that if the effect of only one sling is observable in hyolaryngeal elevation, then all landmarks representing features of the hyolaryngeal complex should have eigenvectors directed towards the common attachment site for that sling (Fig. 36). If both slings have an effect, then landmarks representing the distal anterior muscular sling attachments sites should approximate toward their proximal attachments and the same should be observed for the posterior muscular sling.

We conducted two morphometric analyses to investigate this question. We first analyzed coordinates of multiple swallows from one young healthy subject. In this way we controlled for the effects of individual differences (gender and size) and demonstrate shape changes attributable to movement of the hyolaryngeal complex. In the second study we recorded coordinates at minimum and maximum hyolaryngeal excursion from eleven young healthy subjects to demonstrate the vectors of shape change in a group of young health subjects.

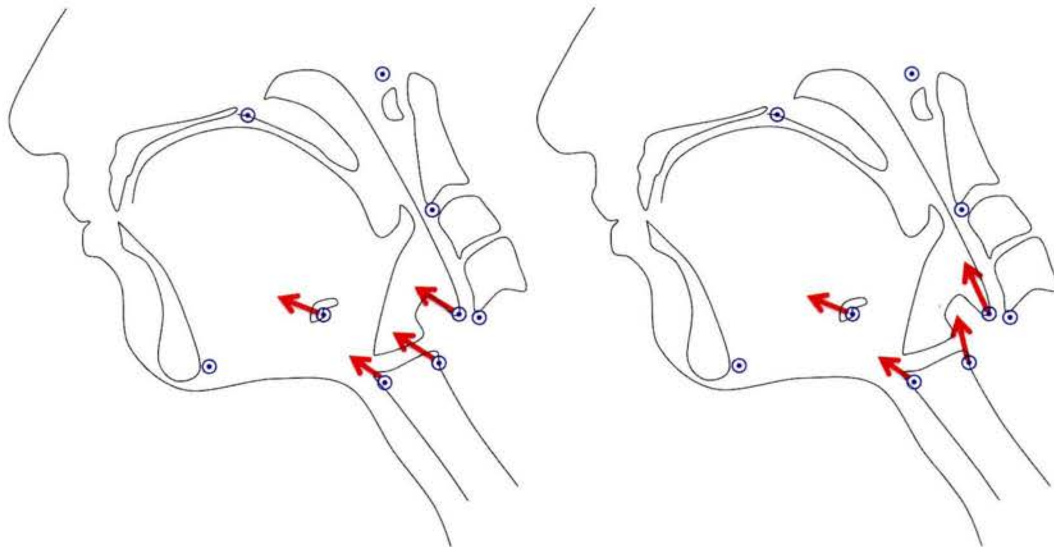

Fig. 36. If only one sling underlies hyolaryngeal elevation, the eigenvectors of coordinates representing the hyolaryngeal complex should have a common direction as pictured on the left (in this case, the vectors for the anterior sling are depicted). If two

slings underlie the system, eigenvectors of coordinates representing the distal attachment of the anterior and posterior muscular sling should diverge as pictured on the right.

Methods

Methods for acquiring dynamic MRI scans of eleven young health subjects while swallowing were described in chapter 5. In that chapter, a method for collecting nine coordinates characterizing the swallowing apparatus was described (Fig. 35). For morphometric analyses of the entire swallowing sequence, coordinates were collected from each frame of dynamic MRI studies of repeated swallows in a single randomly selected subject using Osirix digital imaging and communication in medicine software (http://www.osirix-viewer.com).

For the second morphometric analysis in the current study, the same nine coordinates were collected at minimum and maximum hyolaryngeal excursion from all subjects. Both analyses of coordinates were executed using MorphoJ software, an integrated software package for morphometric analysis including principal component analysis (PCA) (Klingenberg, 2011).

For the first PCA study, we evaluated shape changes within a single subject to control for between-subject differences (Okada et al., 2011). As the temporal resolution of this dynamic MRI acquisition is much lower than videofluoroscopy, we used multiple repeated swallows in this study. In this series the subject swallowed 10 times. Landmark coordinates were collected from every frame, or dynamic, at 4.3 fps during a 10-swallows sequence (49 frames). Each dynamic was classified according to bolus location and phase of oropharyngeal swallow. Oral phase 1 was defined as the bolus in the anterior oral cavity, similar to the hold position in videofluoroscopic studies (Leonard et al., 2000). Oral phase 2 was defined as the bolus in the mid to posterior oral cavity, representing tongue propulsion prior to triggering pharyngeal swallowing. These operational definitions overlap with what has been called early and late stage II bolus

transport in feeding (Hiiemae and Palmer, 1999). Pharyngeal phase 1 was defined as bolus in the hypopharynx during hyolaryngeal elevation. Pharyngeal phase 2 was defined as pharyngeal clearance with the bolus passing through the upper esophageal sphincter. The breakdown for each classification was as follows: 15 dynamics (individual images in a series) for oral phase 1, 12 dynamics for oral phase 2, 12 dynamics for pharyngeal phase 1, and 10 dynamics for pharyngeal phase 2.

The following were performed on the coordinates collected in each of the 49 dynamics. Using MorphoJ, a Procrustean fit was performed on the coordinates to adjust for rotation and difference in scale. Then, a covariance matrix of Procrustean coordinates was generated to compare the shapes. Classifiers describing the phase of swallowing (Oral phase 1 or 2, or Pharyngeal Phase 1 or 2) were assigned to each set of coordinates. A principal components analysis of this covariance matrix was executed. Results of this part of the study include eigenvalues of principal components, shape change of the first principal component as modeled by eigenvectors of each landmark, and a scatter plot of the first two principal components coded by phase of bolus transport.

The second PCA study evaluated shape changes of all subjects at minimum and maximum hyolaryngeal excursion (11 subjects; 2 frames per subject; 9 coordinates per frame) to demonstrate the effects of the two-sling mechanism in a group. Oral phase 1 and pharyngeal phase 1 represent minimum and maximum excursion, respectively. We used coordinates collected in the previous kinematic study with verified reliability (independently collected by two investigators with an inter-rater reliability of r=0.98-1.00). After a Procrustean fit of all coordinates, a covariance matrix of Procrustean coordinates was generated. Classifiers for this study included gender and phase of swallowing; these were assigned to each set of nine coordinates. A principal components analysis of this covariance matrix was executed. Results of this part of the study include eigenvalues of all principal components, shape change of the first principal component as

modeled by eigenvectors of each coordinate, and a scatter plot of the first two principal components coded by minimum and maximum hyolaryngeal excursion.

Results

Principal component analysis of repeated swallows of a single subject including all phases of swallowing resulted in a first principal component (PC) with an eigenvalue representing 81.5% of the variance (Fig. 37). A scatter plot of PC1 and PC2 scores coded by phases of swallowing show that deviations of the grand mean are grouped by phase of swallowing on the PC1 axis indicating that excursion of the hyolaryngeal complex underlies the variance of PC1 (Fig. 38). Predictably, maximum hyolaryngeal elevation, occurring at pharyngeal phase 1, is on one side of *x*-axis at zero; and minimum hyolaryngeal elevation, occurring during the oral phases, is on the opposite side indicating that these two excursion points explain the variance of PC1. Eigenvectors of the first PC for each coordinate are reported in Table 7. Shape changes, as determined by eigenvectors of each coordinate associated with hyolaryngeal excursion (principal component 1) are illustrated in Fig. 39.

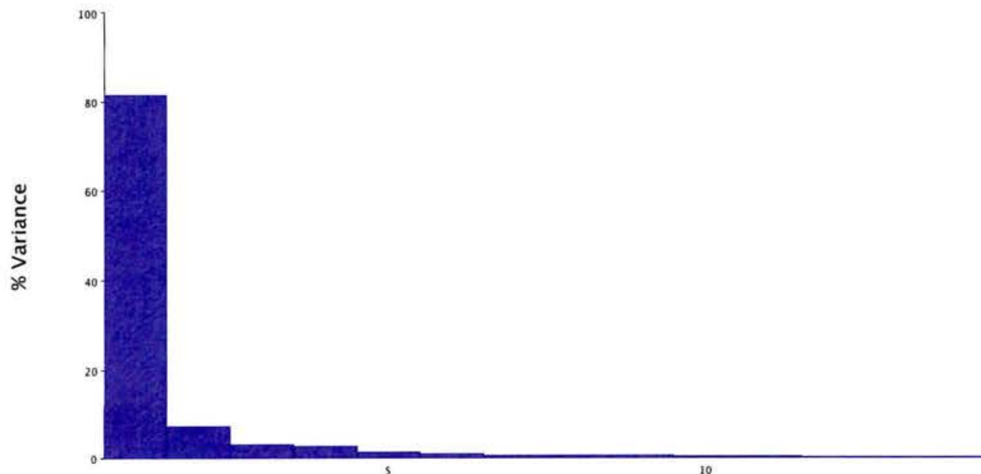

Fig. 37. The first principal component of variance in this analysis defines 81.5% of the shape change variance for principal component analysis of one subject repeated swallows trial.

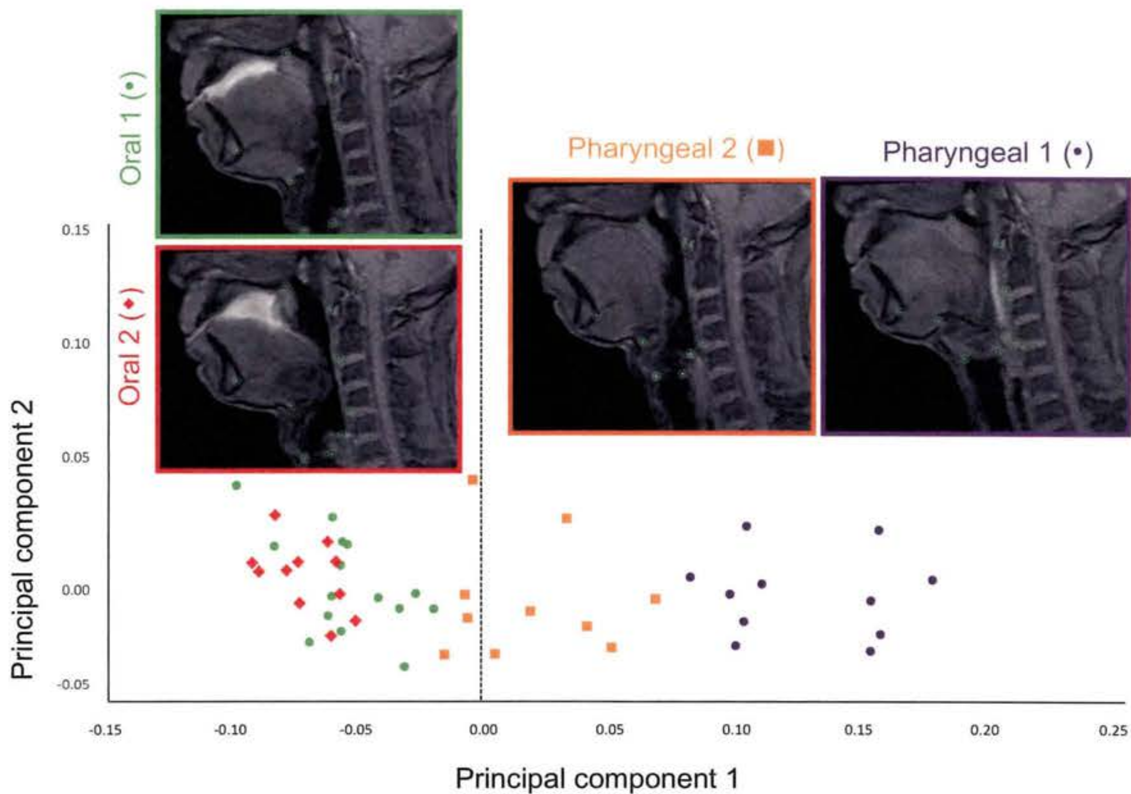

Fig. 38. A scatter plot of PC scores (deviations of the grand mean) for each frame of swallowing coded by phase of swallowing shows that principal component 1 is largely defined by hyolaryngeal excursion. This scatter plot shows oral phase 1 and 2 at one end of the spectrum and pharyngeal phase 1 at the other indicating that these phases represent the extremes of hyolaryngeal excursion. The morphology of the swallowing apparatus in Pharyngeal 2 (bolus has passed into the esophagus before hyolaryngeal descent) is closest to the mean of all PC scores.

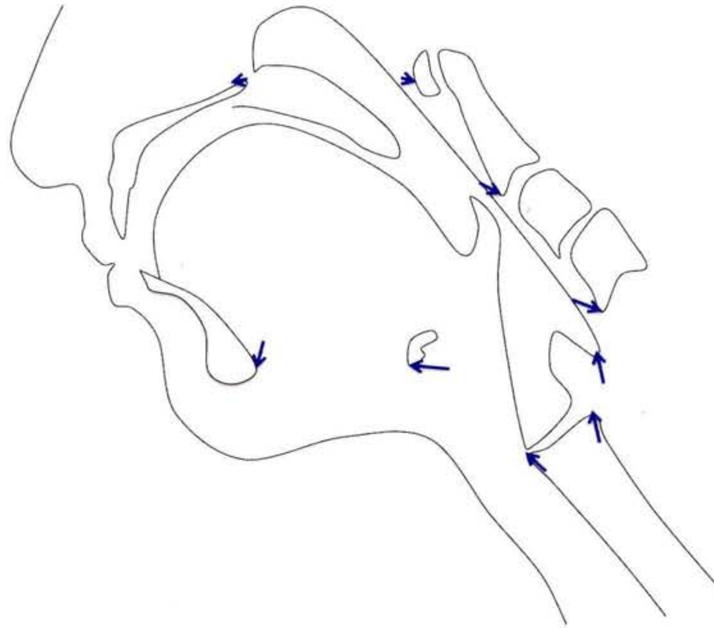

Fig. 39. Shape change (eigenvectors of coordinates) associated with hyolaryngeal elevation (principal component 1) of one subject repeated swallows principal components analysis. These shape changes show that both muscular slings are active in hyolaryngeal elevation.

The PCA of minimum and maximum excursions of the hyolaryngeal complex in all subjects combined resulted in a first PC with an eigenvalue representing 58.8% of the variance (Fig. 40). A scatter plot of PC1 and PC2 scores coded by minimum (oral phase 1) and maximum (pharyngeal phase 1) positions show that excursion of the hyolaryngeal complex underlies the variance demonstrated in PC1 shape changes (Fig. 41). Eigenvectors of PC1 for each landmark range is reported in Table 7. Shape changes associated with these eigenvectors are illustrated in Fig. 42.

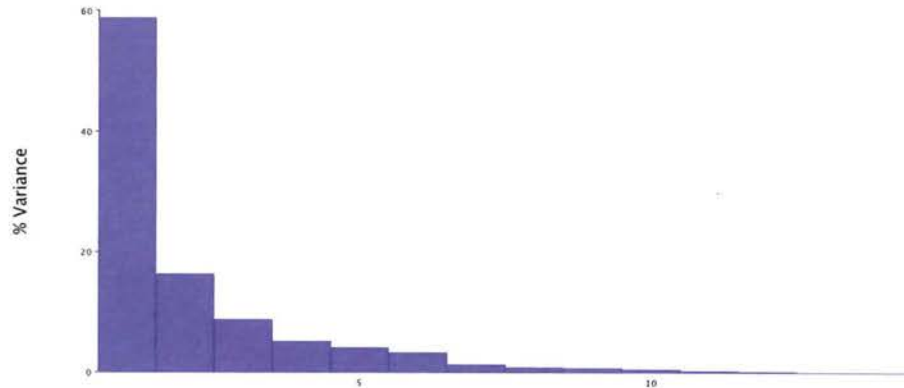

Fig. 40. The first principal component of variance in the between subjects analysis defines 58.8% of the shape change variance

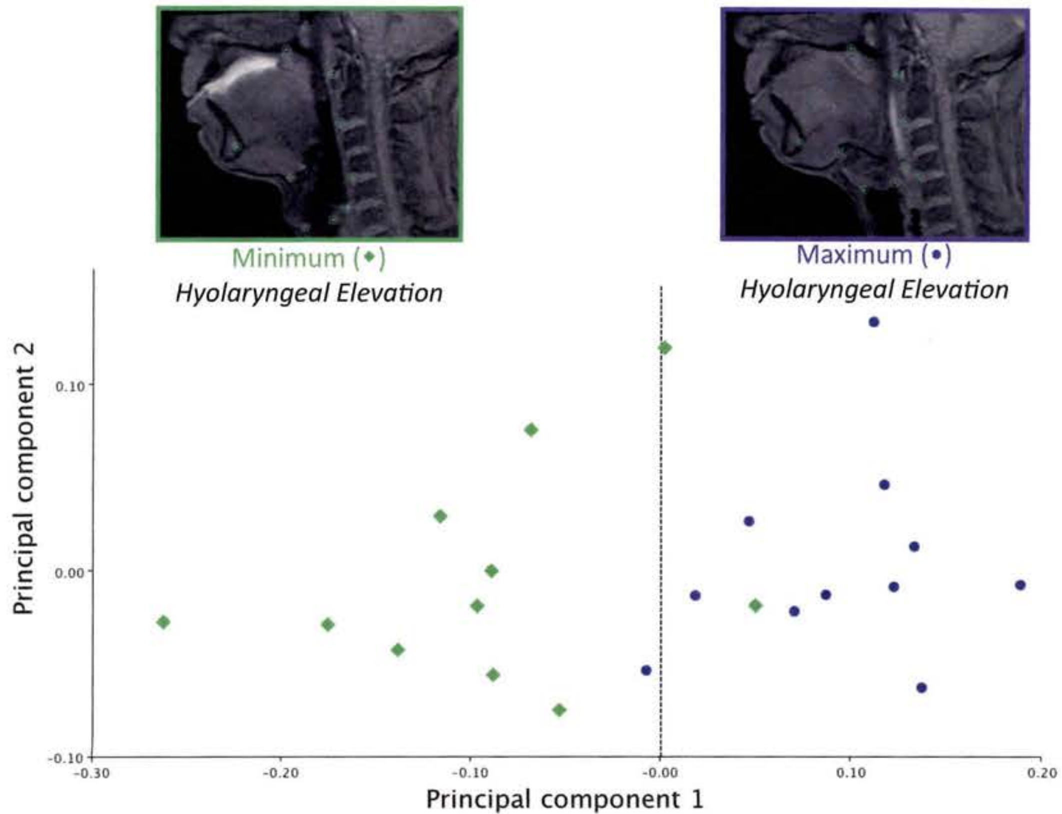

Fig. 41. A scatter plot of summed shape change coded by maximum (pharyngeal 1) or minimum (oral 1) position of hyolaryngeal complex of the between subject analysis shows that principal component 1 is principally defined by hyolaryngeal excursion.

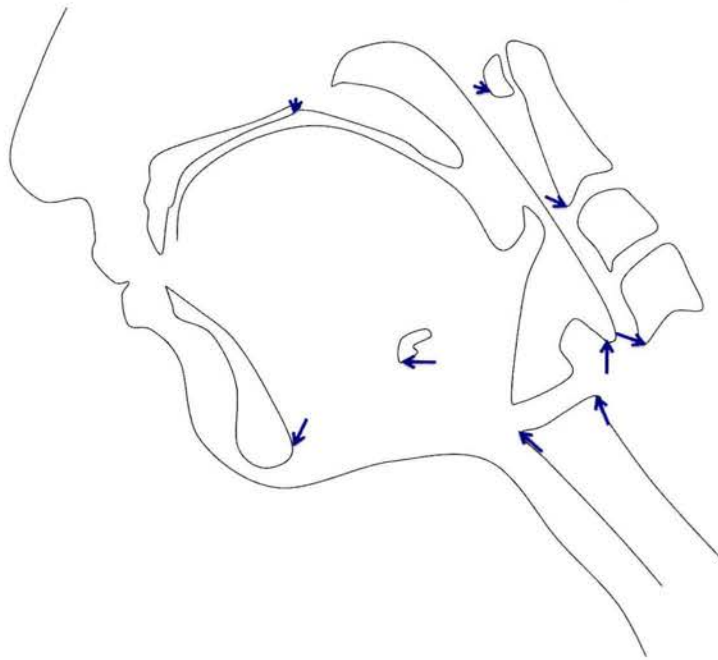

Fig. 42. Shape change (eigenvectors of coordinates) associated with hyolaryngeal elevation (principal component 1) of one subject repeated swallows principal component analysis. These shape changes show that both muscular slings are active in hyolaryngeal elevation.

Table 7. Eigenvectors are here reported from the first principal component of the single subject repeated swallows and the between subject swallows. Eigenvectors indicate the direction of variance in the covariance matrix for each landmark with a common origin of (0,0) set for each eigenvector. A magnitude for each vector was calculated using Pythagoras' theorem. The magnitude should not be construed as relative distance of shape change, rather a reflection of the distribution of covariance in shape change. Greater magnitude reflects a tighter distribution of covariance meaning that shape changes were less random.

	Eigenvectors of single subject repeated swallows			Eigenvectors of between subject hyolaryngeal excursion		
Landmark	x-coordinate	y-coordinate	Magnitude of resulting vector	x-coordinate	y-coordinate	Magnitude of resulting vector
1 (mandible)	-0.09	-0.34	0.35	-0.17	-0.24	0.29
2 (hard palate)	-0.09	-0.04	0.10	-0.05	-0.15	0.16
3 (axis)	0.15	-0.03	0.15	0.13	-0.20	0.24
4 (C2)	0.30	-0.15	0.33	0.31	-0.21	0.37
5 (C4)	0.48	-0.23	0.53	0.37	-0.17	0.41
6 (hypopharynx/UES)	-0.09	0.36	0.37	0.07	0.34	0.35
7 (posterior larynx)	-0.05	0.25	0.26	-0.09	0.34	0.35
8 (anterior larynx)	-0.18	0.17	0.25	-0.15	0.30	0.34
9 (hyoid)	-0.43	0.01	0.43	-0.41	0.00	0.41

Discussion

In this study we were able to observe two functional groups of muscles, and associated skeletal elements, working together to elevate the hyolaryngeal complex. Eigenvectors of landmarks representing the distal attachment of the anterior sling (hyoid) were oriented towards their proximal attachments (mandible), and the landmarks representing the distal attachment of the posterior sling (hypopharynx and posterior commissure of the vocal folds) were oriented towards their proximal attachments (hard palate the cranial base represented by the tubercle of the axis) (Figs. 39, 42).

These morphometric findings indicate a two-sling mechanism underlying hyolaryngeal elevation in swallowing among young healthy adults; that is that the anterior and posterior muscular slings function, not as individual muscles, but as components of a system (Fig. 43). These findings are consistent with shape changes anticipated by anatomical, functional, and kinematic studies of the anterior and posterior muscular slings (Pearson et al., 2012).

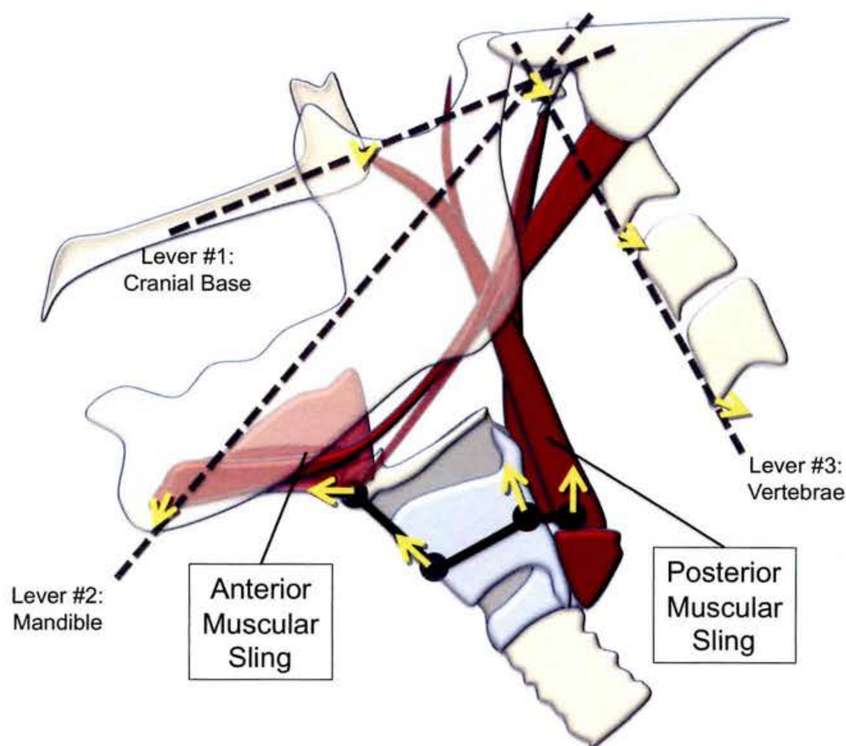

Fig. 43. A diagram (in gray) of the two-sling mechanism, consisting of dashed lines and a solid line connecting large dots, is superimposed over an illustration of relevant anatomical structures. The three dashed lines represent skeletal levers including the cranial base (contiguous with the hard palate), mandible, and vertebrae mapped by 5 coordinates (mandible, posterior edge of hard palate, tubercle of the atlas, C2, C4). The solid gray line connecting large dots represents interconnected features of the hyolaryngeal complex mapped by four coordinates (hyoid, anterior commissure of the vocal folds, posterior commissure of the vocal folds, and the location of the upper esophageal sphincter). Each coordinate maps an element of the two-sling mechanism. Each of the yellow arrows indicates the relative magnitude and direction of covariation of each coordinate during swallowing in a cohort of young healthy normal subjects.

This study documents the covariant shape changes of a dynamic system, something that cannot be appreciated in conventional kinematic studies. While elements of the hyolaryngeal complex are in motion, so are the elements of the three skeletal levers. Eigenvectors of coordinates representing the cervical vertebrae in this study indicate movement of the vertebrae during swallowing (Figs. 38, 41). Anecdotally, the number patients complaining of swallowing difficulty after cervical spine surgery may signify the importance of these skeletal levers to swallowing function. To date, no studies have documented the importance of these levers in relationship to deglutition or dysphagia. Future studies of swallowing mobility could use this method to investigate if swallowing disorders have an impact on the skeletal elements, or if insults to the skeletal elements of the system have an impact on swallowing.

Approximation of the thyroid cartilage to the hyoid has conventionally been attributed to the thyrohyoid muscle alone (Cook et al., 1989). The structure and function of the stylopharyngeus as well as the distinct eigenvector at the posterior commissure of the vocal folds suggests that the thyroid cartilage is in part approximated to the hyoid by the posterior sling muscle. However, the observed eigenvector of the anterior commissure (vocal folds) coordinate towards the hyoid bone is consistent with the line of action representing the thyrohyoid muscle (Pearson et al., 2012). Landmarks located the anterior and posterior commissures of the vocal folds diverge slightly and are not parallel in either of the PCA (see the numerical eigenvectors of landmarks 7 and 8 in Table 7). This may indicate distinct effects of the thyrohyoid elevating the thyroid cartilage toward the hyoid bone and the stylopharyngeus inserting on the posterior border of the thyroid cartilage, also elevating the larynx towards the cranial base represented by the axis. It may also represent the action of the cricothyroid muscle tensing the vocal folds to protect the airway and simultaneously lengthening the vocal folds.

Three main limitations of this study include low temporal resolution, the fact that three dimensional shape changes are here analyzed in two dimensions, and the fact that subjects were swallowing in the supine position. Temporal resolution of this dynamic MRI sequence (8.3 fps) is much lower than videofluoroscopy (30 fps). However, since MRI is a radiation free imaging modality, many more swallows can be recorded without risk to the subject, especially if normal healthy subjects are being studied. While the two-dimensional shape change analysis used in the current study is more translatable to common use videofluoroscopy, a more robust investigation of the distinct elements of shape change in swallowing lies in three-dimensional coordinate data. Three-dimensional coordinate data could be collected with 320-detector-row multi-slice computed tomography (Inamoto et al., 2011). However, multi-slice CT also exposes subjects to radiation. As for the posture of a subject, manometry studies showed no difference between subjects swallowing in the supine versus upright position and may suggest that structure is largely unchanged (Barkmeier et al., 2002). However, more study is needed to know how subject posture affects shape changes as measured by coordinate data of anatomical landmarks.

Significant to this study is the novel use of morphometrics in characterizing swallowing. Rather than measuring multiple variables of movement, this methodology analyzes covariant shape changes of a dynamic system. Components of the system are modeled by linking various coordinates that represent underlying anatomical relationships. There are many potential applications of this method. The consequences of insult to the structural or neural substrate of one or more functional muscle slings may be observable using this method. Common etiologies of dysphagia may result in discernable patterns of shape change. Compensatory strategies or exercise regimens could also analyzed to design treatment plans to address particular insults to the swallowing system.

In conclusion, this study used principle component analysis of multiple swallows in a single subject and hyolaryngeal excursion points in multiple subjects to demonstrate

shape changes of the swallowing apparatus as modeled by coordinates of anatomical landmarks. These shape changes show the effect of a two-sling mechanism elevating the hyolaryngeal complex.

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Chapter 7

The Clinical Relevance of the Two-Sling Mechanism for Hyolaryngeal Elevation: a Videofluoroscopic Study of Post-Treatment Head and Neck Cancer Patients

Abstract

Introduction: Evidence for a two-sling mechanism for hyolaryngeal elevation has been presented in the preceding five chapters of this dissertation. What remains to be determined is if this mechanism is important for facilitating safe and effective swallowing. In this retrospective study measurements taken from videofluoroscopic swallowing studies from a test group were compared with a control group to determine if poor swallowing outcomes are associated with changes in the two-sling mechanism. Swallowing outcomes including penetration-aspiration and pharyngeal residue, kinematics of the anterior and posterior slings, and covariant shape change of the two-sling mechanism of the two groups are compared. Pharyngeal constriction was measured as a possible confounding contributor to poor swallowing outcomes.

Methods: Post-treatment head and neck cancer patients determined to have swallowing difficulty by a clinician served as a test group (*n*=21). This group was age and gender matched with patients complaining of swallowing difficulty but demonstrating "normal" swallowing ability as determined by a clinician as a control group (n=21). Swallowing outcome measurements were quantified using the Penetration-Aspiration Scale and the Normalized Residue Ratio Scale. Anterior muscular sling (AMS) kinematic measurements included time and distance of hyoid excursion during minimum to maximum hyolaryngeal movement in swallowing. Posterior muscular sling (PMS) kinematic measurements included time and distance of posterior thyroid approximation to the cranial base during minimum to maximum hyolaryngeal movement in swallowing. Covariant shape change of coordinates representing the two-sling mechanism was compared using Canonical Variate Analysis. Mahalanobis distances (which function like

z-scores in multivariate analysis) between test and control group subjects were compared. Vectors of covariant shape change (eigenvectors) for each coordinate in the test and control groups were illustrated. Pharyngeal constriction ratio was also measured to determine if weak pharyngeal constriction is also associated with poor swallowing outcomes.

Results: The test group, when compared to the control group, was characterized by poor swallowing outcomes as determined by Penetration-Aspiration Scale ($p<0.0001$) and the Normalized Residue Ratio Scale for the valleculae ($p<0.002$) and piriform sinus ($p<0.003$). Timing and distance measurements of the anterior muscular sling were not significantly different in the two groups, whereas for the posterior muscular sling time of displacement was abbreviated ($p=0.002$) and distance of excursion was reduced ($p=0.02$). A canonical variate analysis showed that the Mahalanobis distances of hyolaryngeal excursion (minimum to maximum hyolaryngeal displacement) for the test group is $D=2.98$, whereas $D=4.54$ for the control group, indicating a greater shape change in the control group during swallowing than in the test group. The covariant shape change for both groups determined by eigenvectors of coordinates indicates a diminished hyolaryngeal elevation and greater neck extension in the test group. The pharyngeal constriction ratio was significantly higher in the test group than the control group ($p=.0001$) indicating less efficient pharyngeal clearance in the test group.

Discussion: In this study we document that swallowing outcomes of post-treatment head and neck cancer patients indicate swallowing difficulties compared to an age- and gender-matched control group. Kinematic measurements indicate a reduced function of the posterior muscular sling muscles in these individuals. Shape changes demonstrate differences in musculoskeletal elements of the two-sling mechanism. A reduced function of the two-sling mechanism is associated with poor swallowing outcomes. Reduced pharyngeal constriction is also associated with poor swallowing outcomes, which may be related to the reduced function of the posterior muscular sling.

Chapter 7

The Clinical Relevance of the Two-Sling Mechanism for Hyolaryngeal Elevation: a Videofluoroscopic Study of Post-Treatment Head and Neck Cancer Patients

Introduction

Reduced hyolaryngeal elevation in swallowing is associated with incomplete clearance of the bolus from the pharynx (Kahrilas et al., 1992). Inability to clear a bolus through the pharynx is a major problem, causing the person to avoid many foods and resort to a diet of liquids. In addition, saliva or food retained in pharyngeal spaces such as the piriform sinuses (residue) may be drawn into the laryngeal vestibule (penetration) and pass through the vocal folds into the trachea (aspiration) after the swallow as the airway reopens. Disordered or diminished movement of the hyoid and larynx is also associated with aspiration during swallowing. Both outcomes are observed among post-radiation head and neck cancer patients (Langmore and Krisciunas, 2010). Two muscular slings that elevate the hyoid, larynx and associated structures have been verified by anatomical and physiological studies in young healthy adults (Chapters 2-6). It is not known if altered function of one or both of these slings is associated with poor swallowing outcomes (penetration, aspiration, or residue). To determine if poor swallowing outcomes are associated with altered function of the two-sling mechanism, we compare kinematic and morphological data collected from videofluoroscopy images of patients presenting with dysphagia (all post-treatment head and neck cancer patients) with age and gender matched controls. The control group was composed of patients who complained of swallowing difficulty, were assessed by a clinician, but were found to have essentially normal oropharyngeal swallowing function (in some cases, esophageal clearance was reduced).

The hyolaryngeal complex is comprised of the hyoid bone, laryngeal cartilages, and associated structures including the cricopharyngeus muscle, which forms the upper

esophageal sphincter. Two muscular slings, with assistance from the thyrohyoid muscle, elevate the hyolaryngeal complex in swallowing (Pearson et al., 2012) (Fig. 44.). The suprahyoid muscles (mylohyoid, geniohyoid, stylohyoid, and digastric) form an anterior sling with proximal attachments to the mandible and cranial base and distal attachments to the body of the hyoid. The distal attachment of this muscular sling on the anterior aspect of the hyoid translates force to the larynx via a fibrous thyrohyoid membrane. A posterior sling, comprised of the long pharyngeal muscles (stylopharyngeus, palatopharyngeus and salpingopharyngeus), has superior attachments to the styloid process, pharyngotympanic tube, and structures associates with the palate. The inferior portions of the palatopharyngeus and salpingopharyngeus intermingle with one another and line the lateral pharyngeal wall. The primary inferior attachment site of the stylopharyngeus is the posterior edge of the thyroid cartilage with a smaller portion of the muscle attaching to the lateral pharyngeal wall. These muscular slings function to elevate the hyolaryngeal complex in young healthy adults, but it is unknown whether pathology changes the function of the two-sling mechanism.

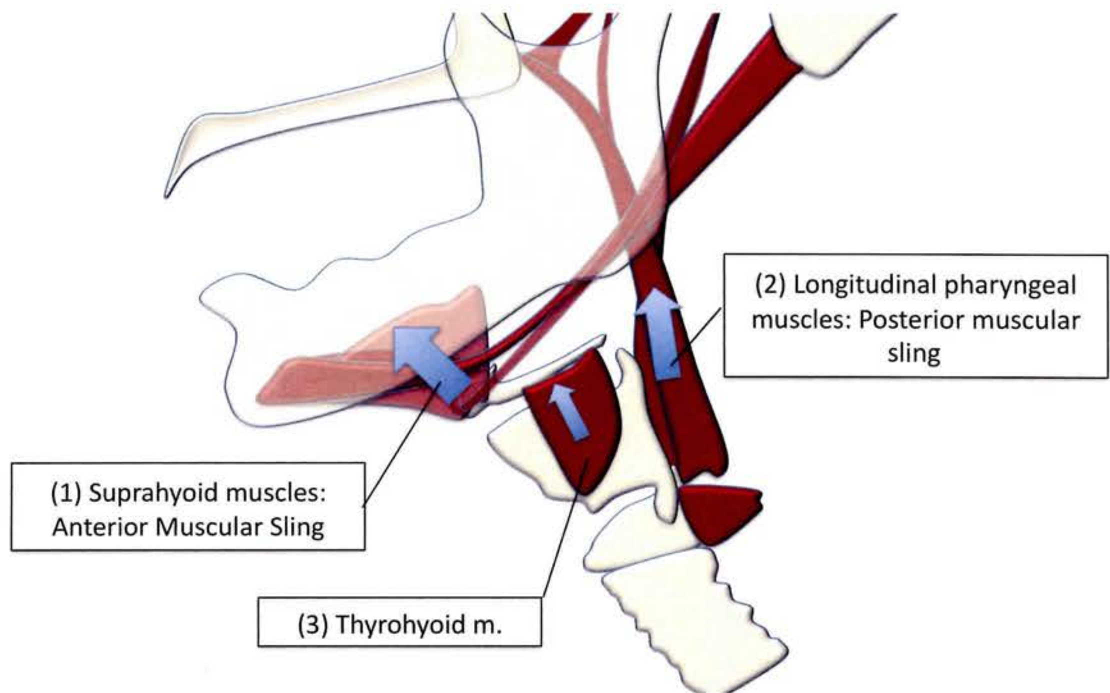

Fig. 44. Two-sling mechanism for hyolaryngeal elevation in swallowing includes the suprahyoid muscles (1) and the long pharyngeal muscles (2). The thyrohyoid muscle (3) is intrinsic to the hyolaryngeal complex.

In this study we use videofluoroscopic studies of swallowing, also known as modified barium swallows (MBS), to collect swallowing outcome variables, kinematic measurements, and coordinates of anatomical landmarks for morphometric analysis. Videofluoroscopic swallow studies involve radiographically imaging patients while they swallow liquid or solid foods. Barium contrast is added to the material being swallowed, thereby allowing a clinician to observe where it passes during the swallow. Some key indicators of poor swallowing include retention of part or all of the bolus in the pharynx (*residue*), *penetration* of barium into the laryngeal vestibule, or *aspiration* of the bolus into the trachea.

Penetration or aspiration of the bolus into the airway may be evaluated using the penetration-aspiration scale, a validated ordinal scale measuring the severity of bolus penetration into the airway (Rosenbek et al., 1996). There is currently no common use standard of measure for residue. The Normalized Residue Ratio Scale, a continuous measurement incorporating the quantity of residue proportionate to the size of the individual and a ratio of residue relative to a particular pharyngeal space (vallecula or piriform sinus) has been proposed (Pearson et al., 2012b).

Using digital analysis, quantitative measurements of structural displacements or timing of events can be obtained to characterize particular events of swallowing (Kendall et al., 2000; Leonard et al., 2000). Measurement of time, duration, or distance of hyoid excursion provides a way of evaluating change in the action of the anterior sling. Duration and distance measurements of the movement of the posterior laryngeal elevation towards the cranial base approximate the action of the posterior muscular sling. However, because the swallowing mechanism is complex, attributing the causes of individual movements is not entirely straightforward. For example, hyolaryngeal approximation is a measurement often attributed to the action of the thyrohyoid (Mepani et al., 2009). However, these studies do not take into account the actions of the longitudinal muscles, which also elevate the thyroid (Cook et al., 1989; Pearson et al., 2012c). Hyoid excursion, hyolaryngeal approximation, and posterior laryngeal elevation are useful for documenting events representing particular features of the two-sling mechanism for hyolaryngeal elevation.

Pharyngeal shortening, a function executed by the longitudinal pharyngeal muscles that elevate the hyolaryngeal complex, is also related to pharyngeal constriction. Pharyngeal constriction is a function of the two layers of the muscular pharyngeal wall (longitudinal pharyngeal muscles and the pharyngeal constrictor muscles). The longitudinal pharyngeal muscles are the interior layer of muscle comprised of slow-twitch muscle fibers (Mu and Sanders, 2007). The pharyngeal constrictor muscles are the external layer

of fast twitch muscle and are more or less oriented orthogonally to the longitudinal muscles. The pharyngeal constriction ratio has been shown to be a reliable surrogate for strength of pharyngeal constriction (Leonard et al., 2011). This ratio measures the area of the hypopharynx represented in lateral view videofluoroscopy at rest and at maximum constriction. This measurement is useful for documenting the effect of the pharyngeal constrictor muscles and the longitudinal pharyngeal muscles.

Confounding the interpretation of particular kinematic measurements of the two-sling mechanism is the fact that these measurements represent elements of an integrated system. Each sling is comprised of multiple muscles with many motor units that may be recruited or inhibited for a particular task. Interpreting a significant change in a single variable may provide insight to a particular feature of swallowing physiology, but does not provide contextual appreciation of changes in a dynamic system with multiple structures. However, the kinematics of the swallowing apparatus can be analyzed as a system using multivariate morphological analysis of anatomical landmark coordinates.

To characterize the system we modeled the underlying structure of the swallowing apparatus as two slings that suspend the free body of the hyolaryngeal complex from multiple levers. To track the position of salient features of hyolaryngeal elevation we chose 9 anatomical landmarks that can be followed to track the movement of three skeletal levers and various features of the hyolaryngeal complex (Pearson et al., 2012a) (Fig. 45). Five coordinates track the movement of the vertebrae, mandible, and cranial base. Four additional coordinates track the movement of the hyoid, anterior larynx, posterior larynx, and the upper esophageal sphincter (sometimes called the pharyngeal esophageal segment). By mapping the positions of these nine coordinates during a swallow, multivariate morphological analysis can be used to evaluate the covariance of each element at minimum and maximum hyolaryngeal excursion (Webster et al., 2010). More specifically, canonical variate analysis is the morphological analysis method used

as it allows for the statistical and visual comparison of variance in hyolaryngeal excursion by classification, such as the test and control group.

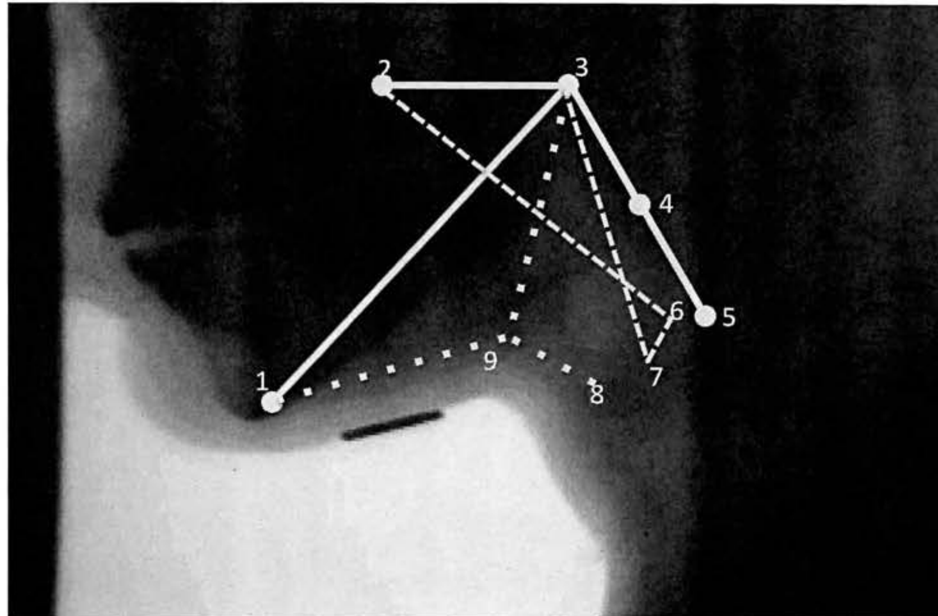

Fig. 45. Five coordinates (1-5) of anatomical landmarks map three relatively rigid levers shown in solid lines (cranial base, mandible, vertebra), and four coordinates (6-9) map elements of the hyolaryngeal complex. Dotted lines represent elements of the anterior muscular sling (suprahyoid muscles and the thyrohyoid) and dashed lines represent elements of the posterior muscular sling (palatopharyngeus and stylopharyngeus).

In this retrospective study, post treatment head and neck cancer patients identified from medical records were designated as a test group (*n*=21). These patients were those who experienced swallowing difficulties and were referred to speech-language pathologists for treatment. Age and gender matched patients judged to have "normal" swallowing ability were designated as a control group (*n*=21). Control group subjects had also been referred to an otolaryngology clinic for swallowing difficulties. Our first aim was to determine if swallowing outcomes differed between these groups hypothesizing that post

treatment head and neck patients have worse swallowing outcomes than the control group, as measured by Penetration-Aspiration and Residue Scales. Our second aim was to determine if kinematic variables reflecting elements of the two-sling mechanism differed between groups, hypothesizing a reduced function in the test group. Finally, we used canonical variate analysis of coordinates mapping the two-sling mechanism to demonstrate differences in shape change during swallowing between the test and control group. We predicted that the covariant elements of the two-sling mechanism would show a greater range of hyolaryngeal excursion in the control group than the test group depicting a more robust function of the two-sling mechanism elevating the hyolaryngeal complex.

Methods

Under a research protocol approved by the Boston University Medical Campus Institutional Review Board, a review of patient records was used to establish a control group and test group for the current study. All of the data in the current study were collected from these patients' modified barium swallows (MBS) imaging studies recorded under routine radiographic protocols approved by Boston Medical Center. A Speech Language Pathologist in collaboration with a Radiologist who manually operated the fluoroscope conducted the MBS studies. During the MBS protocol an attempt was made to attach a radiopaque marker to each subject as an external scalar. Images were produced by a GE Precision Fluoroscopic unit and recorded digitally by a computer workstation at 30 frames/second. QuickTime™ software was used to trim each imaging study to include one episode of cued lateral view 5ml swallows of thin liquid barium solution.

Ninety-three patients seen by otolaryngology with MBS studies were identified. All etiologies of dysphagia related to post treatment head and neck cancer (HNC) were placed in a group (*n*=28) and patients complaining of swallowing difficulty but were assessed as either having no to mild dysphagia ('normal') were placed in another group

(n=45). Of the 28 subjects in the HNC group, two lacked scalars and five could not be age or gender matched with the 'normal' group. This left a final cohort of 21 subjects each in the test and control groups. In each group 7 were female and 14 male. The mean age and standard deviation of the test group was 63.86±13.17 years old, and the control group was 63.00±11.37 years old. To evaluate size differences, a C2-C4 measurement (distance from the anteroinferior corner of the second cervical vertebral body to the anteroinferior corner of the fourth cervical vertebral body) was taken as a proxy for body size. A two-tailed t-test indicated no significant difference in C2-C4 distance between groups (control: 3.22±0.64 cm, test 3.53±0.38 cm).

Swallowing outcomes, spatial, and temporal data were collected from video files using Image J image analysis software equipped with QuickTime™ plug-ins (http://rsbweb.nih.gov/ij). Raters blinded to the group assignment analyzed video files. Reliability was tested for all measurements by using a second judge to re-measure variables in 50% of MBS studies. Interrater reliability is reported in Table 8. In swallowing episodes requiring more than one swallow to clear the bolus, measurements were taken of the first swallow.

Table 8: Inter-rater reliability of each swallowing outcome and kinematic measurement is determined by Intraclass correlation coefficients.

	Intraclass Correlation Coefficient	*Lower 95% confidence limit*	*Upper 95% confidence limit*
Penetration-Aspiration Scale (PAS)	0.87	0.75	0.93
Valleculae Residue ($NRRS_v$)	0.93	0.85	0.97
Piriform Residue ($NRRS_p$)	0.91	0.82	0.96
Anterior Sling Distance Measurement (AMS_d)	0.87	0.75	0.93
Posterior Sling Distance Measurement (PMS_d)	0.88	0.77	0.94
Anterior Sling Time Measurement (AMS_t)	0.88	0.74	0.95
Posterior Sling Time Measurement (PMS_t)	0.90	0.77	0.96
Pharyngeal Constriction Ratio (PCR)	0.81	0.66	0.90

Swallowing outcomes focused on penetration and aspiration of the bolus during swallowing and the amount of residue after swallowing (Steele et al., 2011). To measure penetration and aspiration we used a 1-8 ordinal scale called the Penetration-Aspiration Scale (PAS) as described and validated elsewhere (Rosenbek et al., 1996). In this scale 1 is considered normal. PAS scores of 2-6 indicate penetration of the bolus into the laryngeal vestibule with higher numbers indicating greater severity of penetration. Scores of 7-8 indicate aspiration. To quantify residue we used the Normalized Residue Ratio Scale (NRRS) for the valleculae and piriform sinuses as described elsewhere (Pearson et al., 2012b). The NRRS is a continuous measurement that incorporates the ratio of residue relative to pharyngeal space (valleculae and piriform sinuses) and the amount of residue scaled by an internal anatomical scalar (C2-C4 measurement described above). Differences in PAS in the two groups were compared using Mann Whitney U

tests, and differences in NRRS for the valleculae and piriform sinuses were compared using two-tailed *t*-tests with Bonferroni correction (α=.05, p<.025).

Kinematic data collected from video files included duration and excursion distance measurements representing the anterior and posterior slings. The movement of the hyoid towards the mandible represented the action of the anterior muscular sling (AMS). The movement of the posterior larynx towards the cranial base represented the action of the posterior muscular sling (PMS). Each AMS and PMS time and distance measurement was measured at minimum and maximum elevation. For the AMS, the minimum position was defined as one frame prior to the first rostral movement of the hyoid after the bolus passes into the hypopharynx. This is commonly referred to as "the first jump of the hyoid". Maximum position for the AMS was defined as where the hyoid reached its most rostral position during the swallow. For the PMS, the minimum position was defined as one frame prior to the first rostral movement of the larynx after the bolus is transferred from the oral cavity to the hypopharynx. Maximum position for the PMS was defined as where the larynx reached its most superior position during the swallow.
Duration of AMS sling activity was defined as the duration of hyoid excursion (from minimum to maximum position), and duration of PMS activity was defined as duration of posterior laryngeal excursion. To determine whether either the AMS or PMS is reduced in function in the test group, duration of action in test and control groups was compared using two-tailed *t*-tests with Bonferroni correction (α=.05, p<.025).

Excursion of the hyoid (representing the action of the AMS) and movement of the posterior larynx toward the cranial base (representing the action of the PMS) were calculated using an anatomical land-marking technique designed to track the components of the two-sling mechanism of hyolaryngeal elevation (Pearson et al., 2012, Chapter 5). In this method, nine two-dimensional coordinates of anatomical landmarks that map three skeletal levers and features of the hyolaryngeal complex are collected at minimum and maximum elevation of hyolaryngeal structures. Coordinates were mathematically

converted into excursion distances of the hyoid and larynx. To determine whether either the AMS or PMS is reduced in function in the test group, excursion distances in both groups were compared using two-tailed *t*-tests with Bonferroni correction ($\alpha=.05$, $p<.025$).

Pharyngeal constriction is also associated with poor swallowing outcomes. To evaluate differences in pharyngeal constriction between test and control groups, the Pharyngeal Constriction Ratio (PCR) was measured using the lateral view MBS studies (Leonard et al., 2011). PCR is a ratio of the area of the hypopharynx at maximum constriction to the area of the hypopharynx at rest. Significance of differences in PCR was determined using a two-tailed *t*-test.

Anatomical coordinates used to calculate kinematic variables were also used to evaluate shape changes associated with hyolaryngeal elevation (Chapter 6). In each videofluoroscopy frame, nine coordinates map the elements of the two-sling mechanism for hyolaryngeal elevation. Our coordinate data set included: nine coordinates for each subject at minimum excursion for the test group and control group; and nine coordinates mapping these same landmarks at maximum excursion for the test group and control group. MorphoJ, software for geometric morphometric analysis, was used to perform a procrustean fit of all coordinates (Fig. 46) (Klingenberg, 2011).

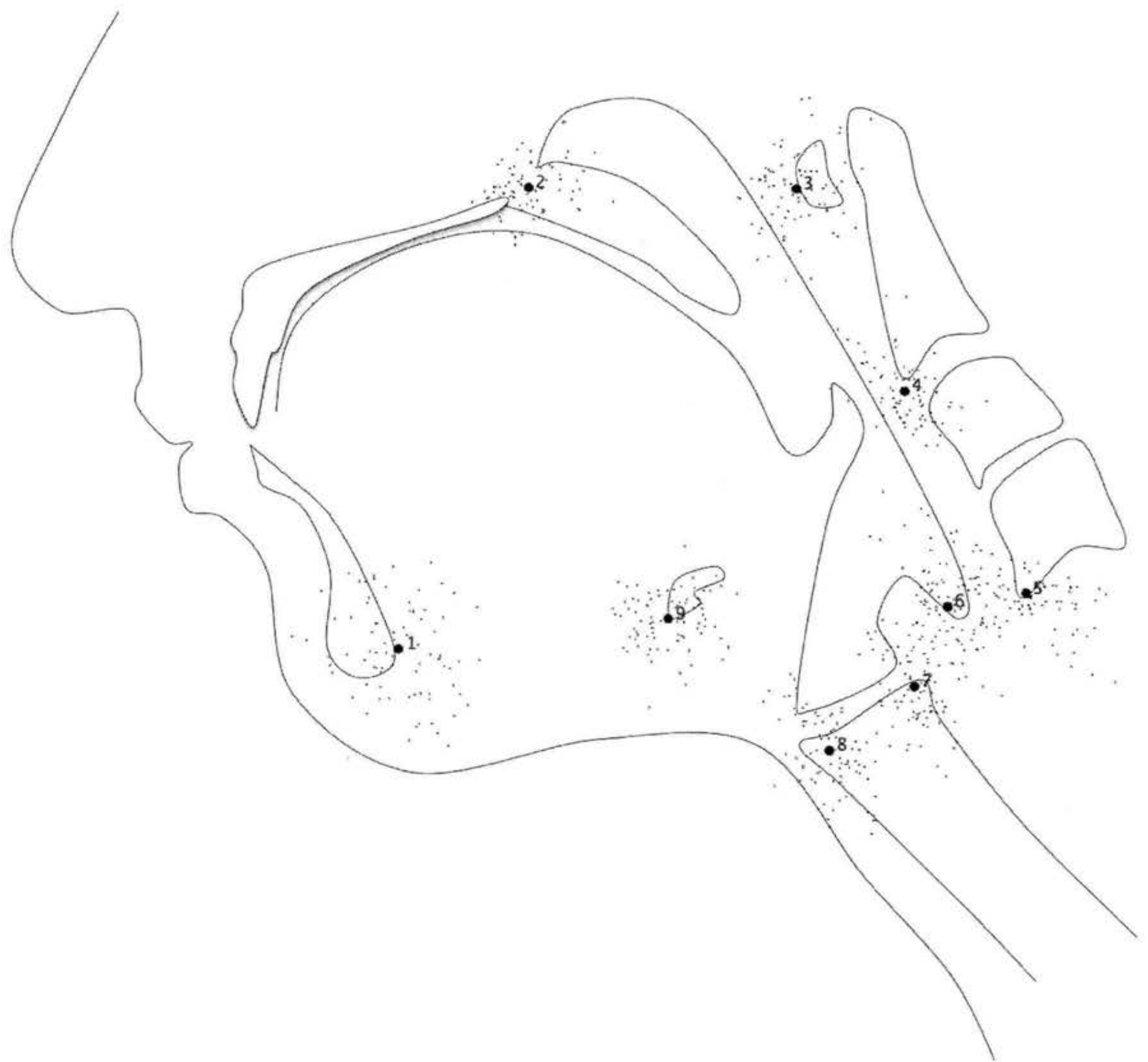

Fig. 46. Procrustean fit of coordinates adjusts for differences in rotation and scale. Here we see coordinates from minimum and maximum positions for all subjects at each of the nine coordinates.

The procrustean fit resolved differences in scale and rotation between all subjects at both excursion points. Following the procrustean superimposition of coordinates, a covariance matrix was generated and used for canonical variate analysis. Canonical variate analysis is a multivariate analysis of covariance similar to principal component analysis, though in this study the data are grouped by *a priori* classification for comparison of mean shape changes by group (Klingenberg and Monteiro, 2005). We grouped sets of 9 coordinates in two classifications: by condition (test and control group) and excursion (minimum and maximum position of the hyolaryngeal complex).

The canonical variate analyses generated several sources of data that were ultimately used to describe shape changes by classification. Each CV was assigned an eigenvalue. Eigenvalues, like beta coefficients, are used to indicate how much of the covariance is explained by each CV. A scatter plot of the first two CV's was generated. In this scatter plot, each overall shape (set of nine coordinates) was given a CV score (Mahalanobis distance) coded by classification variable (control group at minimum excursion, control group at maximum excursion, test group at minimum excursion, test group at maximum excursion) to visually demonstrate relationships between classifications and CVs. The classification variable that most thoroughly distinguishes the data in the scatter plot is considered to be the primary source of variation in those data. A CV score, or Mahalanobis distance, is a standardized dimensionless quantity that describes the overall shape change variance of a subject against the mean of all shape change in the sample. It functions similarly to a z-score in univariate analysis indicating how many standard deviations an observation lies from the mean. A smaller *D*-score indicates a configuration that is closer to the mean, which we interpret as less overall shape change.

Within each canonical variate in this study, eigenvectors were assigned to each of the nine coordinates to indicate the direction of shape change for each coordinate within a CV. Eigenvectors were graphed with wireframes comparing the excursion of the hyolaryngeal complex in the test and control groups. We predicted that the control group

would have greater Mahalanobis distance (*D*-scores), indicating a bigger difference in covariant shape change attributable to more robust action of the two-sling mechanism in hyolaryngeal elevation. We also predicted shape change differences as represented in eigenvector wireframes between control and test group would indicate reduced function of the two-sling mechanism of hyolaryngeal elevation.

Results

All swallowing outcome variables indicated significantly worse swallowing in the head and neck cancer (HNC) group than in the control group. Means and standard deviations and Mann Whitney U test results of Penetration-Aspiration Scale (PAS) scores were as follows: HNC (4.43±2.42), control (1.29±0.56), U=34.5, z=4.67, p<0.0001. Means, standard deviations, and two-tailed t-test results of Normalized Residue Ratio Scale for the valleculae ($NRRS_v$) were: HNC (0.22±0.20), control (0.05±0.11), p=0.002. Means, standard deviations and two-tailed *t*-test results of Normalized Residue Ratio Scale for the piriform sinuses ($NRRS_p$) were: HNC (0.31±0.36), control (0.05±0.14), p=0.003.

Time and distance measurements representing the posterior muscle sling (PMS) demonstrated significant differences between the HNC and control group, but these measurements did not differ statistically for the anterior sling (AMS) (Table 9) (Fig. 47). In other words, there was no significant difference in hyoid movement, but there was a significant difference in the approximation of the larynx to the cranial base, which represents the actions of the posterior muscular sling.

Pharyngeal constriction ratio, representing the anterior-posterior narrowing of the lumen due to the action of pharyngeal constrictors and the superior-inferior shortening of the pharynx due to the action of long pharyngeal muscles, was greater in the test group (Table 9). Means, standard deviations, and two-tailed t-test results of pharyngeal constriction ratio were: HNC (0.16±0.11), control (0.05±0.04), p=0.0001.

Table 9. Means, standard deviations, and *p*-values for time and distance measurements of the AMS and PMS and the pharyngeal constriction ratio (PCR).

Measurement	Control group mean and SD	Test group mean and SD	*p*-values
Time of hyoid movement representing the AMS (seconds)	0.35±0.24	0.23±0.11	0.06
Time of posterior laryngeal movement representing the PMS (seconds)	0.48±0.23	0.29±0.13	0.002
Distance of hyoid movement representing the AMS (cm)	0.93±0.53	0.78±0.47	0.36
Distance of posterior laryngeal movement representing the PMS (cm)	1.41±0.73	0.89±0.63	0.02
Pharyngeal constriction ratio	0.05±0.04	0.16±0.11	0.0001

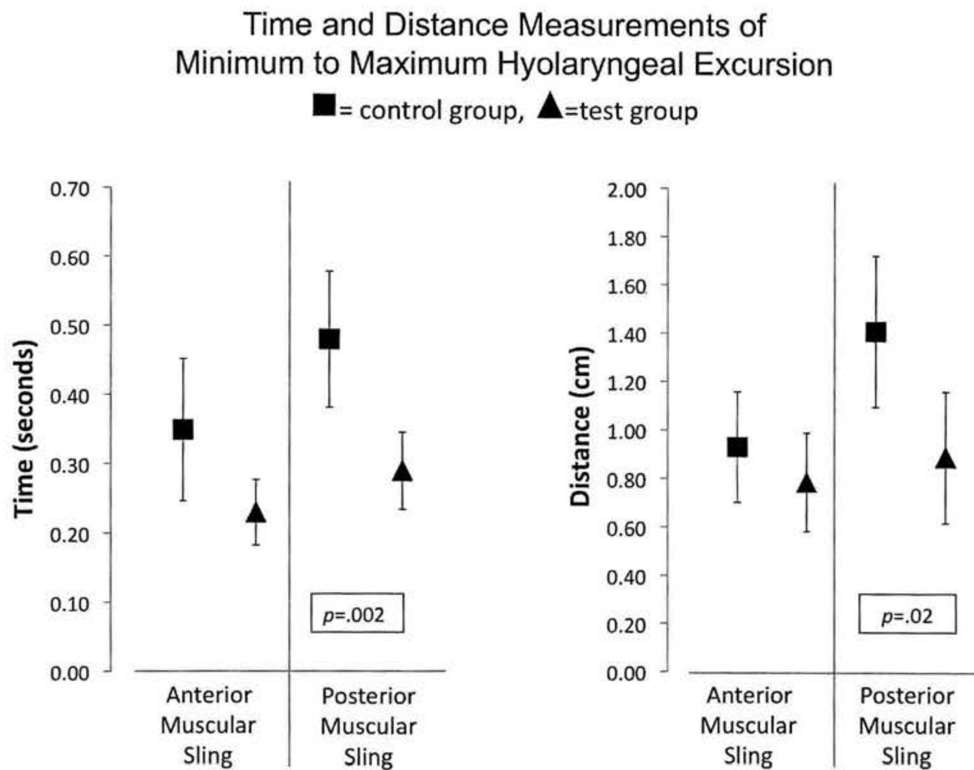

Fig. 47. Significant differences were found between control and test groups for the posterior muscular sling in both time and distance measurements of minimum to maximum hyolaryngeal movement in the pharyngeal phase of swallowing.

The scatter plot of canonical variate scores, or Mahalanobis distances, indicated that CV1 is associated with the excursion of the hyolaryngeal complex and CV2 is associated with condition (the test and control groups) (Fig. 48). Mahalanobis distances (*D*) between the test group (post-treatment head and neck cancer patients) and control group ('normals') at minimum excursion=1.98, whereas at maximum excursion *D*=2.76. Mahalanobis distances of hyolaryngeal excursion (minimum to maximum) for the test group is *D*=2.98, whereas *D*=4.54 for the control group. *D* values, like a z-score, show us the generalized distance from the mean. Here, a greater *D*-score means a greater shape

change, whereas a lesser *D*-score means that the shape is relatively unchanged. Eigenvectors for each coordinate graphed on a wireframe demonstrate the direction and degree of covariant shape change of the control (Fig. 49) and test (Fig. 50) groups for visual comparison. In these graphs a reduced laryngeal elevation and greater extension of the head and neck can be observed in the test group.

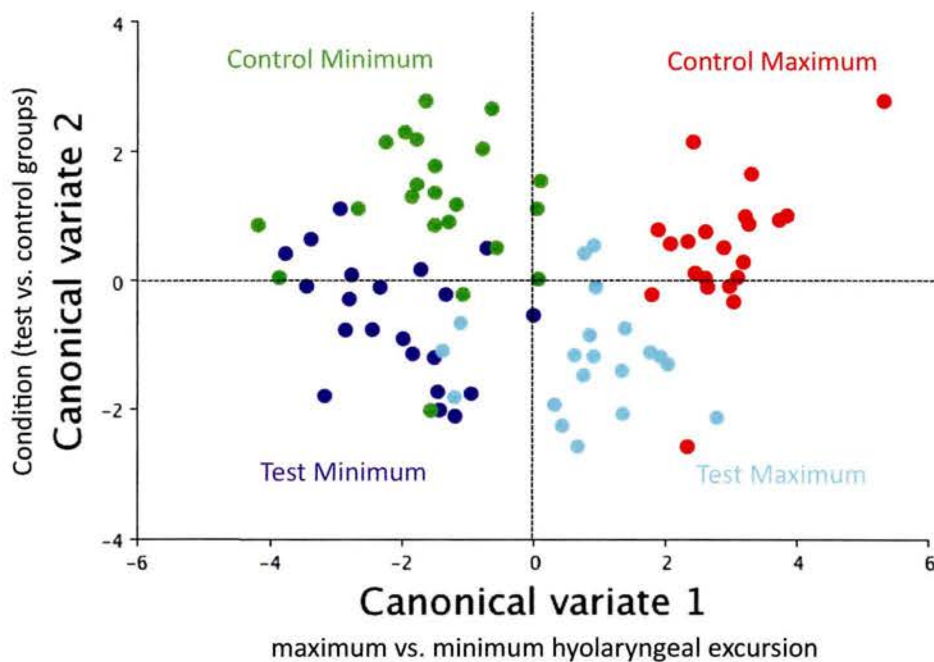

Fig. 48. Mahalanobis distances (CV scores) of overall shape change for each subject are plotted by classification. Excursion of the hyolaryngeal complex (maximum vs. minimum excursion) is associated with CV1, which accounts for 81% of the total variance. Condition (Head and Neck Cancer vs. Controls) is associated with CV2, which accounts for 17% of the variance. Controls, Maximum = the control group of subjects at maximum

hyolaryngeal position; Controls, Minimum= the control group of subjects at minimum hyolaryngeal position; HNC, Maximum=Head and Neck Cancer (test group) at maximum hyolaryngeal position; HNC, Minimum=Head and Neck Cancer (test group) at minimum hyolaryngeal position.

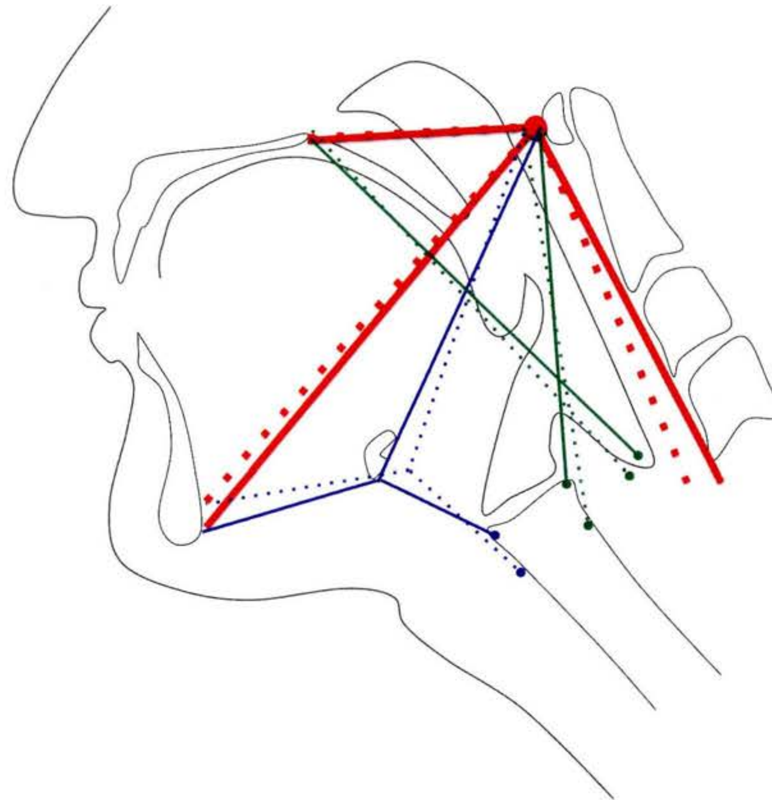

Fig. 49. Eigenvectors indicating shape changes in hyolaryngeal elevation of the control ('normal') group. Dotted lines indicate mean shape at minimum hyolaryngeal excursion, and solid lines indicate the mean shape at maximum hyolaryngeal excursion. Thick red lines mark the changing position of the three skeletal levers (vertebrae, mandible, and cranial base). Dark blue lines mark the action of the anterior muscular sling (suprahyoid muscles) to elevate the hyolaryngeal complex. Light green lines mark the action of the posterior muscular sling (palatopharyngeus and stylopharyngeus) to elevate the hyolaryngeal complex..

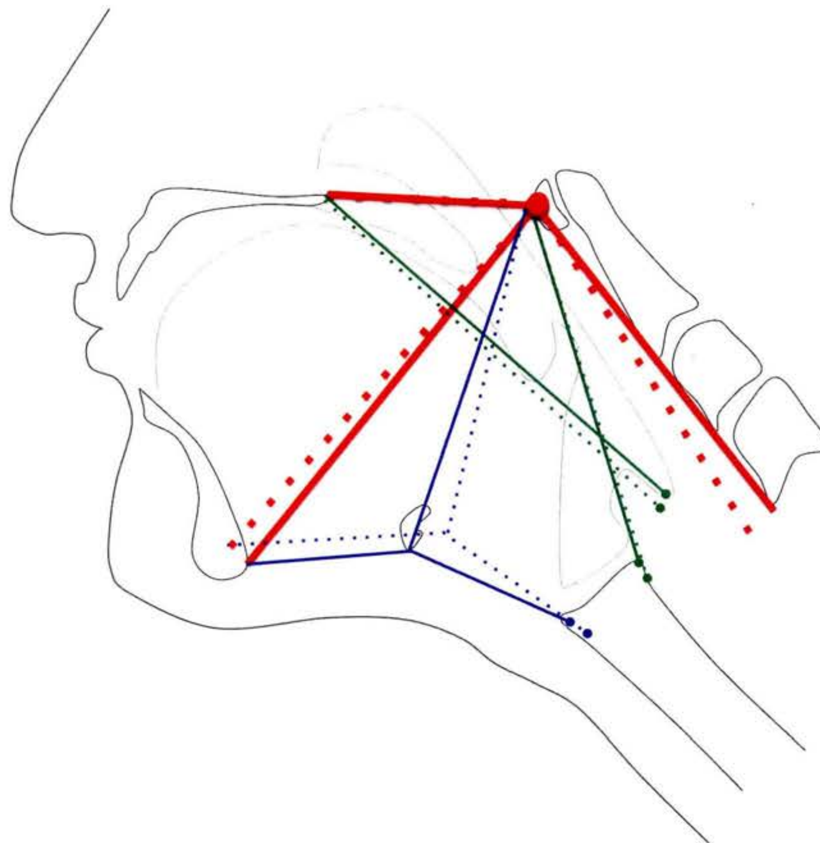

Fig. 50. Eigenvectors indicating shape changes in hyolaryngeal elevation of the post-treatment HNC group. Dotted lines indicate mean shape at minimum hyolaryngeal excursion, and solid lines indicate the mean shape at maximum hyolaryngeal excursion. Thick red lines marking the position of the three skeletal levers (vertebrae, mandible, and cranial base) show a greater extension of the head and neck in the test group compared to the control group. Dark blue lines marking the anterior muscular sling (suprahyoid muscles) and thyrohyoid muscle show a similar hyoid excursion but a reduced laryngeal elevation. Light green lines marking the posterior muscular sling (palatopharyngeus and stylopharyngeus) show a reduce elevation compared to the control group.

Discussion

This study shows a significant difference in functional outcomes, kinematic variables, and shape changes between post-treatment head and neck cancer patients and patients assessed for swallowing difficulty and judged to have 'normal' swallowing function. Specifically, the supposition that post-treatment head and neck cancer patients have poorer swallowing outcomes as measured by penetration-aspiration and residue was confirmed. Kinematic analyses indicate a significant difference between the groups in time and distance measurements representing the posterior muscular sling function, confirming the proposition that reduced function of at least one sling is associated with poor swallowing outcomes. Canonical variate analysis of the two-sling mechanism for hyolaryngeal elevation depicts a difference between groups in overall shape change including a reduced laryngeal elevation and greater extension of the head and neck.

Swallowing outcomes

Poor swallowing outcomes among post-treatment head and neck cancer patients, including increased penetration-aspiration and residue, are consistent with other findings in the literature (Feng et al., 2007; Gillespie et al., 2004). However, this is the first time that the Normalized Residue Ratio Scale has been used to quantify residue in head and neck cancer (HNC) patients. These findings confirm that the test and control groups used here are appropriate for a retrospective pseudo-experimental design for investigating underlying mechanisms of dysphagia, specifically focusing on the role of the two-sling mechanism of hyolaryngeal elevation.

Kinematic measurements

Reduced hyolaryngeal elevation and hyoid movement has been noted among HNC patients (Kendall et al., 1998; Pauloski et al., 2006), and reduced hyoid and laryngeal motility have also been associated with penetration-aspiration (Bingjie et al., 2010; Steele et al., 2011). Association of the structures that underlie laryngeal elevation with poor

swallowing outcomes has not been reported. Our data indicate a diminished function of the posterior muscular sling among HNC patients in both time and distance measurements. This is consistent with findings in the radiation oncology literature that the longitudinal pharyngeal muscles (posterior muscular sling) presumed to lie within the pharyngeal constrictor region of interest in computed tomography scans are more important than t he anterior sling of muscles to swallowing function (Eisbruch et al., 2004; Feng et al., 2007). However, this does not agree with other published studies of post-treatment HNC patients showing decreased hyoid displacement (Kendall et al., 1998). A limitation of our current study is that our sample size is small and the location of the lesions and focus of radiation treatments in the HNC patients were not accounted for. The importance of the anterior muscular sling involvement must not be ruled out. The consensus in the literature is that the suprahyoid muscles (anterior muscular sling) along with the thyrohyoid elevate the hyolaryngeal complex (Cook et al., 1989; Matsuo and Palmer, 2008). However, the results of this study indicate that the posterior muscular sling appears to have a clinically significant role, suggesting that a two-sling mechanism of hyolaryngeal elevation is important to effective swallowing.

It is important to note that we did not include hyolaryngeal approximation, the movement of the anterior aspect of the larynx towards the hyoid bone, in this study. This movement is thought to be the unique function of the thyrohyoid muscle (Mepani et al., 2009). In a cohort of young health subjects the thyrohyoid was not found to be consistently active (Chapter 4). It has also been shown that the stylopharyngeus attaches to the larynx and elevates the thyroid cartilage toward the hyoid bone (Meng et al., 2008; Pearson et al., 2012, Chapter 3). Conversely, it could be argued that the thyrohyoid (if active) assists the approximation of the posterior larynx towards the cranial base, which would in turn confound our PMS measurement.

Analysis of the complex as a system

Deglutition is a dynamic process of interrelated structures that covary in function, a fact that complicates all kinematic studies of swallowing. Rather than focusing on individual variables related to swallowing dysfunction, we propose that a more informative approach is to analyze changes to the system as a whole. We have introduced morphological analysis of coordinates that map three skeletal levers and the connected elements of the hyolaryngeal complex that we call the two-sling mechanism of hyolaryngeal elevation. We found that greater variance in shape changes in hyolaryngeal elevation is a marker for more favorable swallowing.

Canonical variate analysis of landmark coordinates showed overall shape changes judged on the basis of the Mahalanobis distance statistic (*D*-score). The Mahalanobis distance, also referred to as a generalized distance, is a dimensionless quantity that indicates how the covariance of one set of variables (in this study, a set of 9 coordinates) differs from the mean. A smaller *D*-score is interpreted as less overall shape change to the swallowing apparatus as mapped by 9 coordinates. We correctly predicted that mean *D*-scores of the control group at maximum excursion were greater than the test group; when the two-sling mechanism is fully functional, the shape of the swallowing apparatus changes more than when it is not fully functional.

More interesting than the overall shape change scores are the eigenvector plots for each group (Fig. 6a-b). By comparing these plots of control and test groups it can be observed that there is little difference in the covariant distance of hyoid movement or pharyngeal shortening. The greatest differences are associated with laryngeal elevation (posterior thyroid to cranial base approximation and hyolaryngeal approximation) and greater extension of the head and neck in the test group. This observation suggests that subjects with reduced laryngeal elevation may attempt to compensate by hyper-extending the neck. These kinds of systemic observations are not possible using kinematic variables only.

Association between kinematics and function

While this study demonstrates that the two-sling mechanism is important to hyolaryngeal elevation, we cannot say that reduced function of the two-sling mechanism is solely responsible for the poor swallowing outcomes. It is also possible that the functional difference between the groups could be explained by the significantly different degrees of pharyngeal constriction as measured by the pharyngeal constriction ratio (PCR) between the test and control groups (p=0.0001). A higher PCR indicates weaker pharyngeal constriction (Leonard et al., 2011). A spearman rank-order correlation of PCR with the penetration-aspiration scale (PAS) for the entire sample was r=0.74, whereas distance measurements of the posterior muscular sling correlated with PAS was r=-0.42, suggesting that poor swallowing outcomes in the post treatment head and neck cancer patients are more strongly correlated with PCR than with posterior muscular sling function. Adding to the complexity of the problem, it is likely that a reduced function of the posterior sling contributes to higher PCR. Leonard and colleagues have suggested that reduced hyolaryngeal approximation, a function that can be related to the posterior muscular sling, is associated with weaker pharyngeal constriction (Leonard et al., 2004). However, another study found that reduced hyolaryngeal elevation was more associated with residue than was pharyngeal pressure (Olsson et al., 1997).

It remains unclear how to best characterize differences in the two-sling mechanism associated with disordered swallowing. While diminished posterior muscular sling function is indicated by kinematic measurements, eigenvectors of coordinates also demonstrate differences in the skeletal elements of the two-sling mechanism (Fig. 6a-b). It might also be insightful to investigate other related functions such as base of tongue movement or arytenoid to epiglottal closure to see if these are associated with reduced hyolaryngeal excursion. Using an anatomical landmarking methodology to collect coordinates to produce both kinematic and morphological analysis allows for a more expansive investigation of effective and disordered swallowing.

In conclusion, this study demonstrates significant differences in swallowing outcomes between post-treatment head and neck cancer patients and 'normal' controls. We demonstrate reduced laryngeal elevation kinematics attributable to deficits in the posterior muscular sling in the head and neck cancer group compared to the control group. Furthermore, multivariate morphological analysis reveals functional differences in the two-sling mechanism associated with pathology including different positioning of skeletal levers in addition to reduced laryngeal elevation. Whether the shape changes in the test group represent compensatory or maladaptive behaviors remains unclear. Kinematic and morphometric analysis each provide methods that could be used to test various strategies to improve swallowing function.

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Chapter 8

The Effect of Aging and Pathology on Functional Integration of the Two-sling Mechanism of Hyolaryngeal Elevation

Abstract

Introduction: The function of the two-sling mechanism has been validated in previous chapters. In the current study, modularity hypothesis testing was used to determine if muscles comprising each muscular sling function as modules. Evaluation of modular covariation was used to determine if the two slings function in an integrated or independent way, and whether age and/or pathology have an effect on modular covariation.

Methods: Coordinate data mapping the two-sling mechanism at minimum and maximum hyolaryngeal excursion generated in Chapter 7 is utilized in the current study. The test group is composed of head and neck cancer (HNC) patients. The control group is composed of age and gender matched patients who demonstrate normal pharyngeal swallowing. These groups are subdivided into younger (<65) and older (≥65) subgroups to create the following cohorts: younger controls (n=10), younger HNC (n=10), older controls (n=10), and older HNC (n=10). Using MorphoJ software, coordinates are partitioned into hypothesized modules representing each sling for evaluation. The posterior muscular sling configuration included the anterior laryngeal coordinate. Random variable (RV) coefficients indicating the covariation of the anterior and posterior slings were determined for each cohort and used to demonstrate the effect of pathology and aging on the two-sling mechanism for hyolaryngeal elevation.

Results: Hypothesized modularity of the anterior and posterior slings was validated for both younger groups. In older cohorts an alternative partition was generated that coupled

the anterior laryngeal coordinate with the anterior sling, whereas the coordinate remained with the posterior sling in younger patients as hypothesized. RV coefficients of muscular sling modularity for each cohort was as follows: younger control group: RV=0.61; younger test group: RV=0.30; older control group: RV=0.68; and older test group: RV=0.41.

Discussion: Modularity hypothesis testing confirmed the modularity of the anterior and posterior muscular sling. The covariation of the anterior laryngeal coordinate differed in younger and older cohorts and may indicate a shift in functional balance from the posterior sling to the anterior sling as the primary elevator of the larynx. In normal controls the two slings demonstrated weak covariation indicating that the two slings function in an integrated manner. Functional integration (covariation) of the two-sling mechanism declined among post-treatment head and neck cancer patients in both younger and older subjects.

Chapter 8

The Effect of Aging and Pathology on Functional Integration of the Two-sling Mechanism of Hyolaryngeal Elevation

Introduction

A two-sling muscular mechanism suspends the hyolaryngeal complex and moves it during swallowing. The hyolaryngeal complex is comprised of the hyoid bone, larynx and attached structures including the cricopharyngeus. The suprahyoid muscles function as an anterior muscular sling, exerting force principally on the body of the hyoid. The longitudinal pharyngeal muscles function as a posterior muscular sling, lifting the thyroid cartilage from behind and shortening the pharynx. The thyrohyoid muscle is intrinsic to the hyolaryngeal complex and presumably functions to approximate the hyoid and the thyroid cartilage. The function and covariance of structures comprising the two-sling mechanism was validated in Chapters 5 and 6. However, these findings do not specifically validate the presupposed elements of each sling as a functional module, nor do they describe the functional relationship of one sling to another. The purpose of this study is threefold: (1) to confirm that the elements of each muscular sling function as distinct modules; (2) to determine the degree of covariation of the anterior and posterior muscular slings and thereby demonstrate the degree of their functional integration; and (3) to determine whether age and/or pathology have an effect on functional covariation of the two-sling mechanism for hyolaryngeal elevation.

Modularity hypothesis testing applied to anatomical landmark data was used to evaluate the modularity of a presupposed partition of coordinates mapping the minimum and maximum position of the two-sling mechanism in pharyngeal swallowing (Klingenberg, 2009). In this test, if the covariance of presupposed subgroups of coordinates (a partition) was lower than all other randomly generated partitions, then it was assumed that the hypothesized partition is indicated. After validating modularity, the degree of

covariance among the modules demonstrates the relative functional integration or independence of the modules. The statistic used in this test is a random variable (RV) coefficient. RV coefficients are interpreted like an *r*-value with '0' indicating no covariation and '1' indicating complete covariation. Covariation is here interpreted as functional integration. By assigning classifications to sets of coordinates, the effect of age or pathology on functional integration (modular covariance) was appreciated by comparing within group RV coefficients.

To address the aims of this study we used coordinates from the control and test groups from Chapter 7. These groups were divided by age into younger (<65yo) and older (≥65 years old) groups. Hypothesized partitions into anterior and posterior muscular slings were tested in each group (Fig. 51). The coordinate of the anterior commissure of the vocal folds (anterior larynx) is contiguous but not a necessary element of either sling. This coordinate was included since the results may indicate a shift in functional balance between the two slings. If it is assumed that the thyrohyoid is not recruited for swallowing (Chapter 4) then the movement of this coordinate should covary with the muscular sling that principally elevates the larynx. If the anterior sling is primarily responsible for laryngeal elevation then the anterior laryngeal coordinate should covary with the anterior sling. If the posterior sling is primarily responsible for laryngeal elevation then the anterior laryngeal marker should covary with the posterior sling. The RV coefficients for each classification group were used to evaluate functional integration of modules for all groups. By comparing RV coefficients of younger and older test and control groups, the effects of aging and pathology on functional integration were demonstrated.

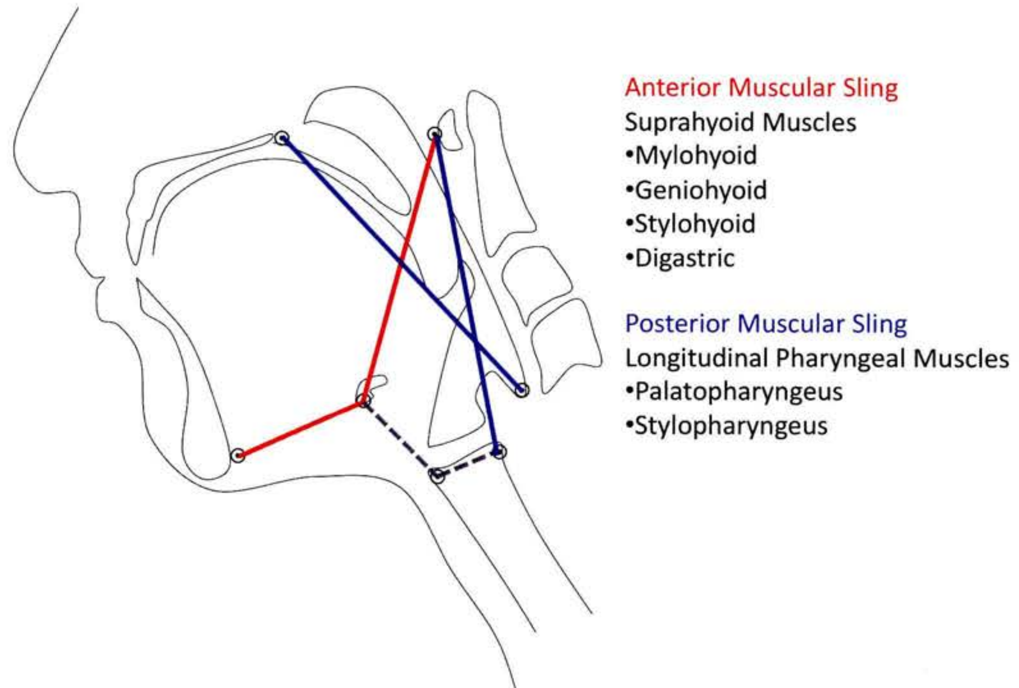

Fig. 51. Red lines connecting coordinates represent the anterior sling module. Blue lines connecting coordinates represent the posterior sling module. The dashed gray lines indicate the possible inclusion of the anterior laryngeal coordinate with either module.

Methods

Designating classification groups

In the previous chapter, 21 post-treatment head and neck cancer (HNC) patients were assigned to a test group. Twenty-one age and gender matched controls were selected from a cohort of subjects judged to have normal function by a speech-language pathologist after videofluoroscopic imaging study (Modified Barium Swallow). The current study further divided test and control groups by age. Groups were subdivided into a younger group (<65yo) and an older group (≥65yo) (Fig. 52). To even groups for

comparison, one control and one test subject was deleted, leaving 10 subjects for each of the 4 cohorts. Group compositions are reported in Fig. 52. Coordinates collected in Chapter 7 map elements of the two-sling mechanism at minimum and maximum hyolaryngeal excursion including three skeletal levers (vertebrae, mandible, and cranial base) and features of the hyolaryngeal complex (hyoid, anterior larynx, posterior larynx, and the pharynx at the upper esophageal sphincter).

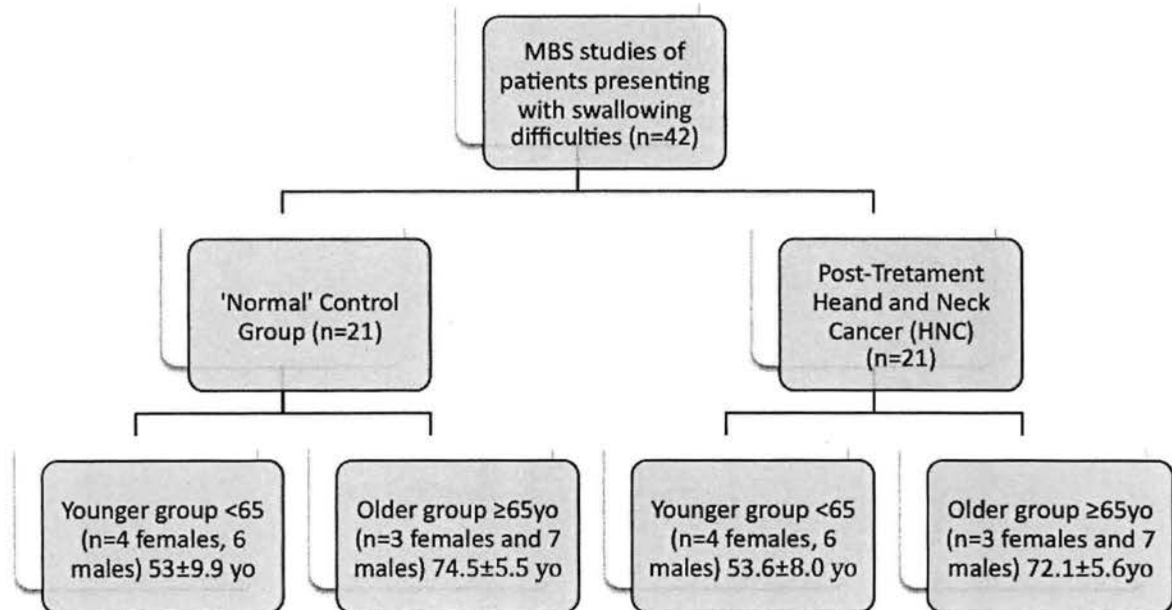

Fig. 52. Group composition including gender breakdown and mean age with standard deviation

Validating hypothesized modularity

The partition of coordinates forming the hypothesized modularity is illustrated in Fig. 53. Three coordinates were assigned to the anterior sling module and four coordinates to the posterior sling module. Two coordinates mapping the vertebral column recorded in Chapter 7 were excluded, as they are not anatomically connected to either muscular sling.

All coordinates were uploaded into MorphoJ software (Klingenberg, 2011). A set of minimum and maximum excursion coordinates of each subject was assigned a classification (younger controls, younger HNC, older controls, older HNC). After a procrustean fit of coordinates (Webster et al., 2010), a covariance matrix of coordinates was generated. Hypothesized configurations of coordinates representing the anterior and posterior slings were partitioned for each classification group with the parameters described above (Fig. 53). The hypothesized modularity was compared with all possible randomly generated partitions of modularity. The partition with the greatest statistical modularity is plotted for comparison with the hypothesized modularity. The end result is that hypothesized modules are validated or an alternative hypothesis is generated and plotted. It should be noted that it is possible to statistically generate modules of covariance that do not make anatomical sense, therefore alternative partitions that are statistically generated should be critically evaluated.

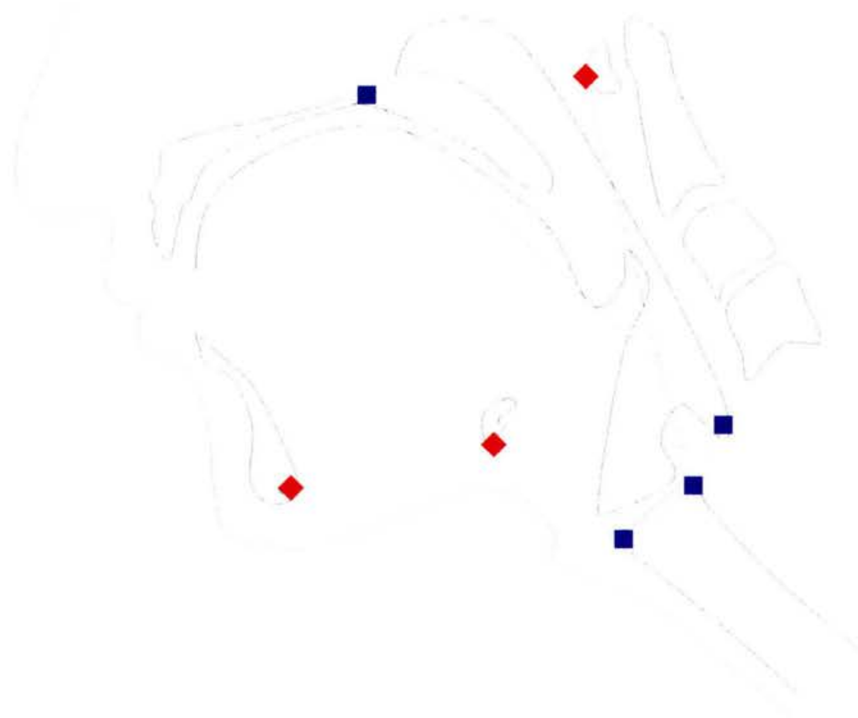

Fig. 53. Hypothesized partition of two-sling modularity in younger control and test groups. Red diamonds represent the anterior muscular sling, blue squares the posterior muscular sling and thyrohyoid muscle. Straight lines represent anatomically connected structures. Here the anterior laryngeal coordinate covaries with the posterior sling. If we assume that the thyrohyoid is not principally a swallowing muscle then these results indicates that the posterior sling is the principal muscular sling elevating the larynx.

Demonstrating functional integration or independence of modules and the effect of aging and pathology

Random variable (RV) coefficients between modules representing the anterior and posterior slings were produced for each group. Higher RV coefficients, indicating stronger covariation between modules, were interpreted as functional integration. Lower RV coefficients, indicating weaker covariance, were interpreted as functionally less integrated modules. RV coefficients for each group are recorded and compared.

Results

Hypothesized modularity

The hypothesized modularity for each of the younger groups (control and HNC) was validated as illustrated in Fig. 53. However, in both older groups (control and HNC), statistical modularity testing determined an alternative partition of modularity (Fig. 54). The difference is with the covariation of the anterior laryngeal coordinate. The coordinate representing the anterior commissure (thyroid cartilage) was functionally covariant with the posterior sling in younger cohorts and functionally covariant with the anterior sling in older cohorts. In all groups, the anterior and posterior muscular slings were validated as separate functional modules.

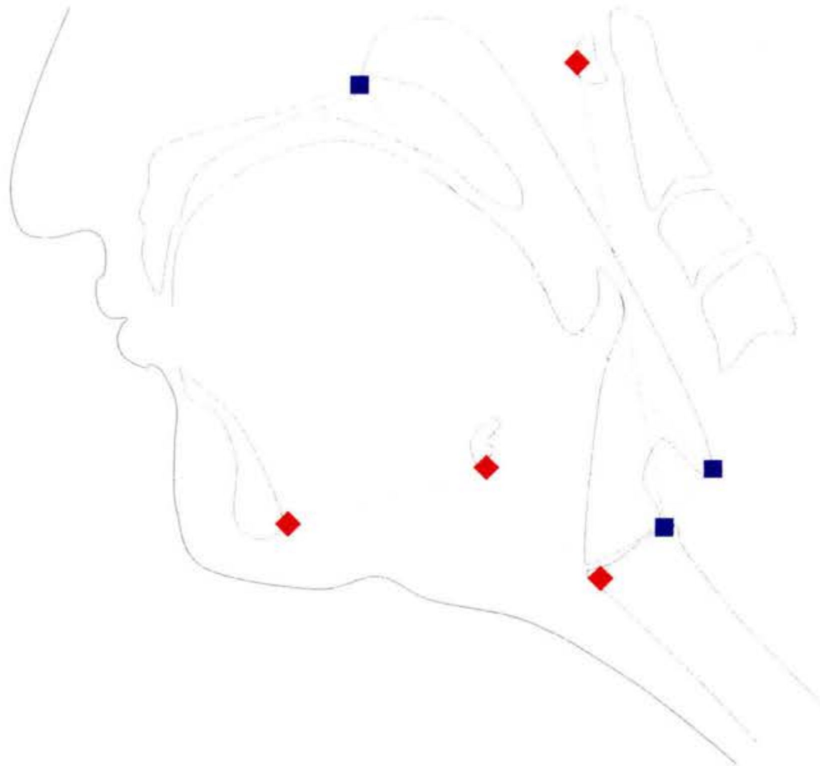

Fig. 54. Alternative hypothesis of modularity determined by randomly generated partitions with the lowest between group covariance in older control and test groups. Red diamonds represent the anterior muscular sling and thyrohyoid, blue squares the posterior muscular sling. Straight lines represent anatomically contiguous structures. Here the movement of the anterior larynx covaries with the anterior muscular sling. This may indicate that the anterior sling is the principally responsible for the elevation of the larynx (traction of the hyoid translates force to the larynx via the fibrous thyrohyoid membrane).

Functional integration of modules and the effect of aging and pathology

Random variable (RV) coefficients for each group were as follows: younger controls RV=0.61, younger HNC RV=0.30, older controls RV=0.68, and older HNC RV=0.41. Therefore, younger and older control groups demonstrated a greater functional integration of both slings than younger and older HNC groups (Fig. 55).

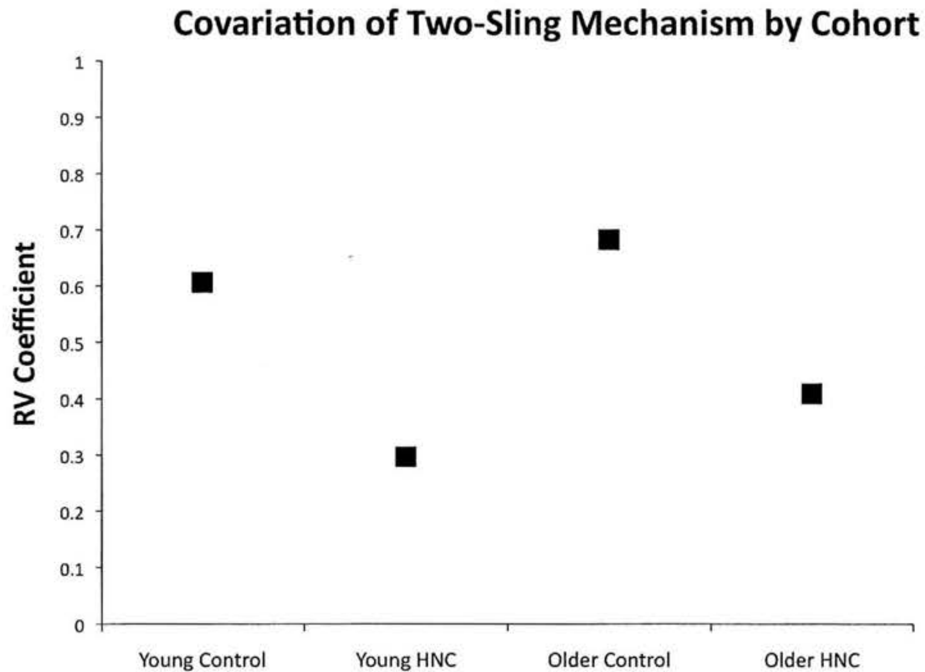

Fig. 55. Random variable (RV) coefficients document the covariation of the anterior and posterior muscular slings for each cohort (Young Control= normal swallowing <65 years old, Young HNC = post radiation head and neck cancer patients < 65 years old, Older Control = normal swallowing ≥ 65 years old, Older HNC = post radiation head and neck cancer patients ≥ 65 years old).

Discussion

The findings of this study indicate that (1) elements of the anterior and posterior muscular slings function as modules; (2) the movement of the anterior laryngeal coordinate covaries with the posterior muscular sling in younger subjects and with the anterior muscular sling in older subjects; (3) the anterior and posterior muscular slings in normal controls are functionally more integrated than independent; and, (4) age does not appear to affect functional integration while pathology does.

Hypothesized modularity evaluated

The hypothesized partition was validated in younger groups and an alternative partition was statistically generated in the older groups. In both partitions, modularity of the anterior and posterior slings was confirmed. The movement of the anterior laryngeal coordinate is the apparent difference between younger (Fig. 53) and older (Fig. 54) cohorts. If the thyrohyoid is not active, as was found in Chapter 4, then the functional covariation of the anterior laryngeal coordinate with a particular sling identifies which sling is principally elevating the larynx. In younger cohorts it appears that the posterior muscular sling lifts the larynx from behind as the primary elevator of the larynx, whereas in older subjects the anterior muscular sling by traction on the hyoid, is primarily responsible for laryngeal elevation. What this may demonstrate is a shift in the balance of function where the anterior sling becomes more important with age. In fect, evidence of increased hyoid excursion has been reported among the elderly (Kendall and Leonard, 2001).

Effect of age and pathology on the modularity of the two-sling mechanism

The primary finding from evaluating the covariation of the anterior and posterior muscular slings was that the slings are less functionally integrated among post treatment head and neck cancer patients than among normal subjects. Reduced integration was demonstrated among both younger and older subjects. In Chapter 7 we documented a

reduction of timing and distance in the excursion of the posterior sling. This is likely what is reflected here since morphometric analysis of coordinates recorded at minimum and maximum hyolaryngeal excursion conflates the effect of space and timing. In addition to characterizing the effects of pathology or treatment on hyolaryngeal elevation using kinematic measurements, it may prove useful to determine the functional covariation of the two-sling mechanism as an indicator of effective or ineffective swallowing.

The limitation of the present study is that the 'normal' subjects populating the control group also presented with complaints of swallowing difficulties. In conclusion it was found that the anterior and posterior slings function as modules, a shift in which sling principally elevates the larynx is associated with aging, and that the insult of cancer or cancer treatment weakens the functional integration of the two-sling mechanism of hyolaryngeal elevation in both younger and older patients.

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Chapter 9

Conclusion

Abstract

This chapter reviews what was accomplished in this thesis to provide evidence of the structure, function, and clinical relevance of the two-sling mechanism of hyolaryngeal elevation in the pharyngeal phase of swallowing. A discussion follows of contributions made to the field of deglutition and dysphagia research. Future directions for continued research are discussed.

Chapter 9
Conclusion

The preceding chapters describe the structure, function, and relevance of the two-sling mechanism for hyolaryngeal elevation in the pharyngeal phase of swallowing. Previous studies have proposed that the suprahyoid muscles (anterior muscular sling) and the long pharyngeal muscles (posterior muscular sling) elevate the structures they suspend, namely the hyoid, larynx and pharynx. The series of studies included in this dissertation provide new evidence coupling the structure of the two muscular slings that suspend the hyolaryngeal complex with the function of hyolaryngeal elevation during swallowing. Established methods were specially adapted for purposes of these studies, and these adaptations have future utility in the field of deglutition and dysphagia research.

Effective and safe pharyngeal swallowing involves directing a bolus through the hypopharynx into the esophagus while protecting the airway and hyolaryngeal elevation is critical to both of these purposes. Pharyngeal swallowing is a complex series of events and several important aspects of the process have not been explored in this work, including: bolus propulsion into the hypopharynx by the action of the tongue, occlusion of the oral cavity by the base of tongue, closure of the nasopharynx by the palatal velum, inhibition of the respiratory diaphragm during expiration (known as swallowing apnea), vocal fold adduction, anterior tilt of the arytenoids to cover the vocal folds, retroflexion of the epiglottis over the arytenoids, inhibition of the cricopharyngeus, and generation of pharyngeal pressure by the pharyngeal constrictor muscles. Each of these events is relevant to effective swallowing. However, the topic addressed in this dissertation, hyolaryngeal elevation, is related to many of these functions including tongue position, epiglottic posture, and pharyngeal squeeze.

In this thesis, we provide evidence to substantiate the structure, function, and clinical relevance of a two-sling mechanism of hyolaryngeal elevation (Fig. 56). In Chapters 2

and 3, structural properties of muscles and charts of muscle orientation were used to describe the structure of the two-sling mechanism. These investigations determined that the two-sling mechanism is structured to elevate the hyolaryngeal complex (Fig. 57a-b). Then whole muscle T2 signal profiles from mfMRI were used to demonstrate the activity of muscles comprising the two-sling mechanism (Chapter 4)(Fig. 58). In Chapter 5 the two-sling mechanism was mapped with coordinates of anatomical landmarks using images from dynamic MRI. These coordinates were used to calculate kinematic measurements demonstrating that the muscles of the two-sling mechanism act to elevate the hyolaryngeal complex (Fig. 59). However, since the hyolaryngeal complex is interconnected, it is possible that the action of suprahyoid muscles could produce the effects attributed to the posterior muscular sling as measured by kinematics. To resolve if both slings are actively elevating, we used morphological analysis of coordinates in Chapter 6. This analysis demonstrated that attachment sites of the anterior and posterior slings move towards one another during swallowing, indicating that they are both active to elevate the hyolaryngeal complex (Fig. 60).

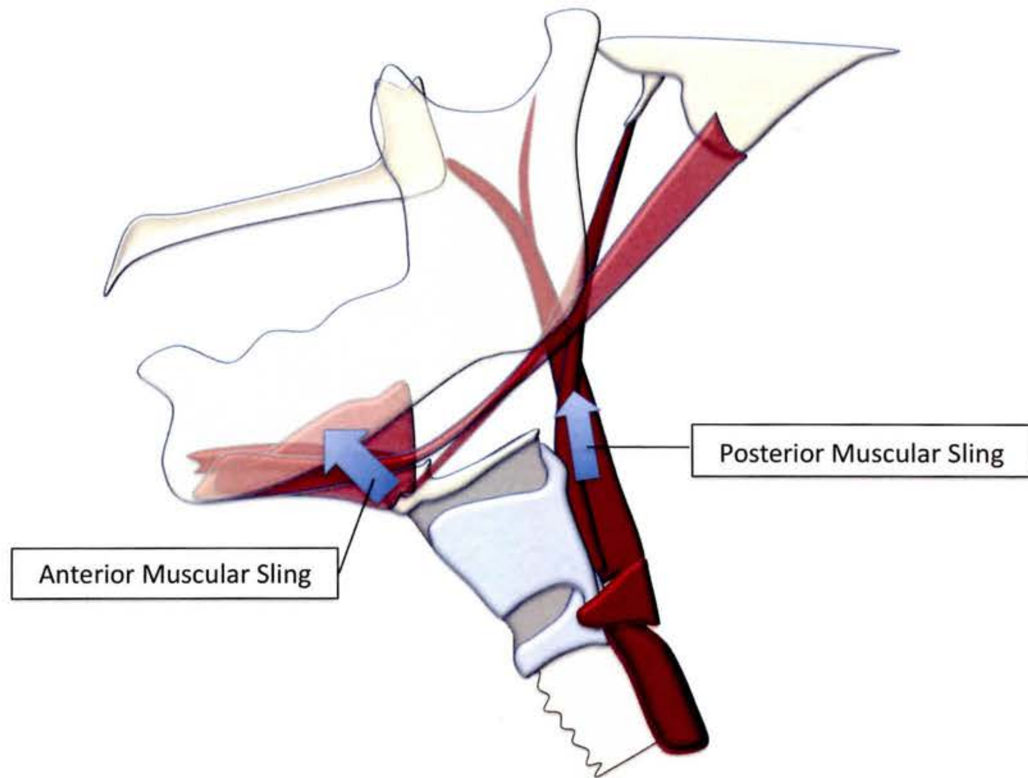

Fig. 56. Proposed two-sling mechanism for hyolaryngeal elevation is illustrated. Hyolaryngeal elevation functions to assist in protecting the airway from bolus penetration into the laryngeal aditus or aspiration thorough the vocal folds into the trachea, and to stretch open a relaxed upper esophageal sphincter.

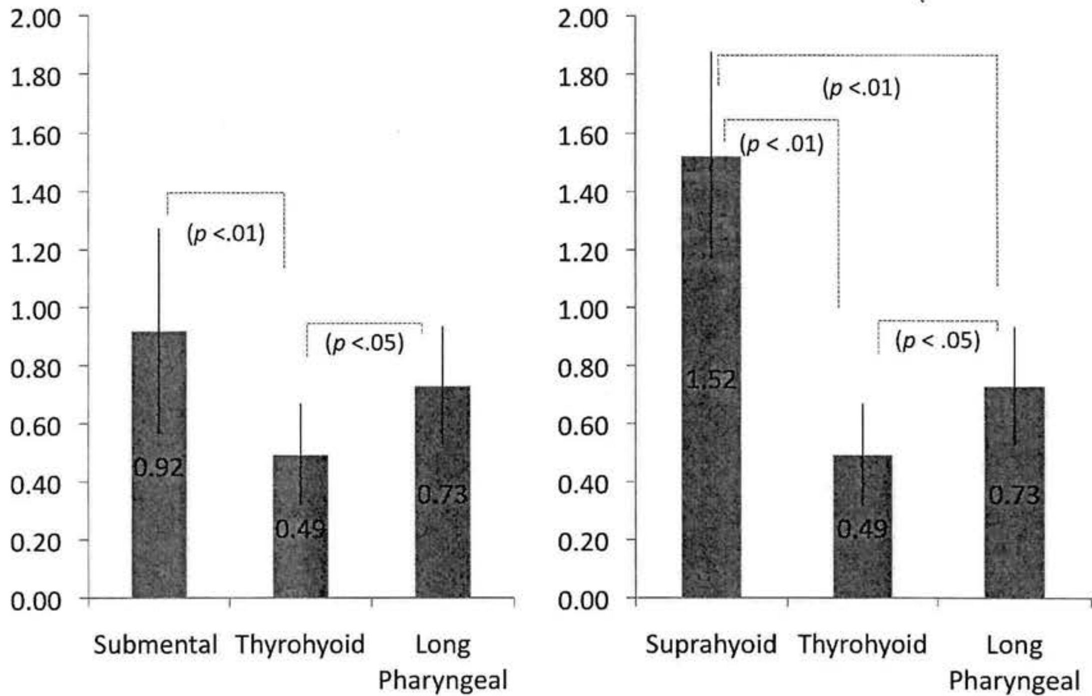

Fig. 57a-b. Means and standard deviations of superior PCSA force unit vectors (cm^2) of: submental vs. thyrohyoid vs. long pharyngeal muscles in Fig. 57a, and of suprahyoid vs. thyrohyoid vs. long pharyngeal muscles in Fig. 57b. An analysis of variance with Tukey HSD of the unit force vector shows that the long pharyngeal muscles have as much potential to elevate the hyolaryngeal complex as the submental muscles, though the suprahyoid muscles as a group have the greatest potential for force in hyolaryngeal elevation.

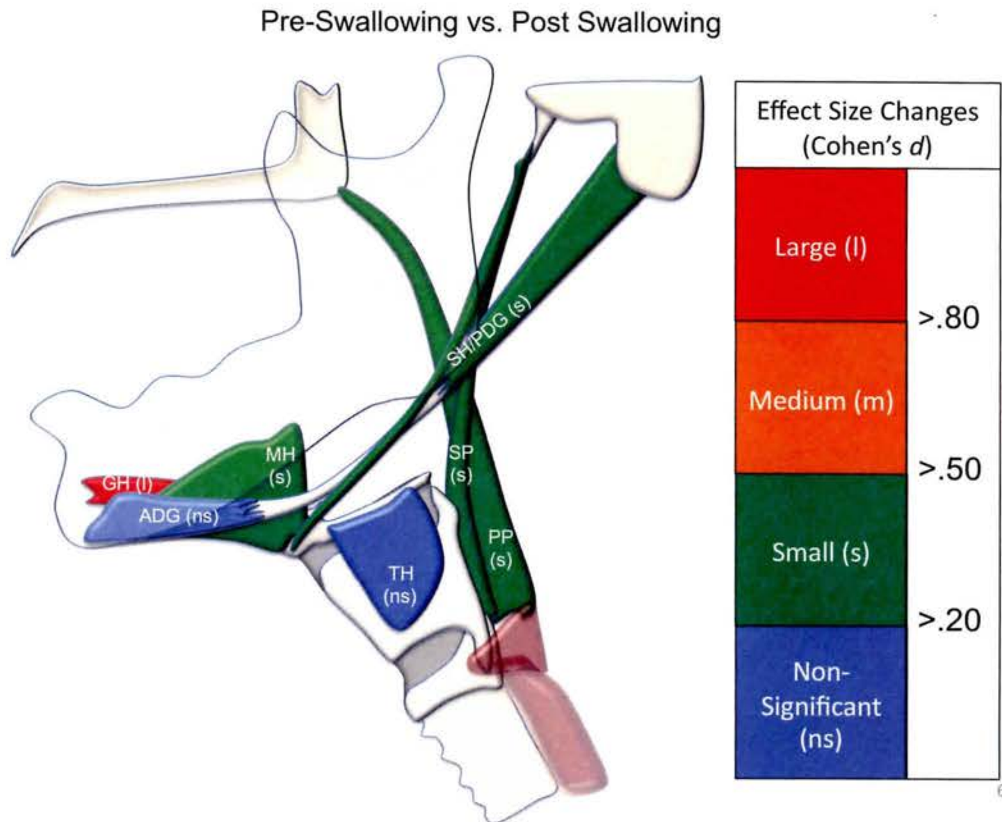

Fig. 58. Pre-swallow vs. post swallow effect size changes (Cohen's *d*) of whole muscle T2 signal profiles depicted for each muscle (GH=geniohyoid, ADG=anterior digastric, MH=mylohyoid, TH=thyrohyoid, SH/PDG=stylohyoid/posterior digastric, SP=stylopharyngeus, PP= palatopharyngeus). Cohen's *d* greater than 0.20 is considered significant with >.50 interpreted as a medium effect size and >.80 as a large effect size change. All muscles excepting the thyrohyoid and anterior digastric achieved significant effect size changes during swallowing. The geniohyoid here demonstrates a large effort, which may be due to the fact that geniohyoid is primarily responsible for the anterior movement of the hyoid here exacerbated by the experimental condition of subjects swallowing in a supine position. The posterior sling muscles, mylohyoid, posterior digastric and stylohyoid all show a small effect size, which is desirable for muscles underlying the repetitive and necessary functions of thriving.

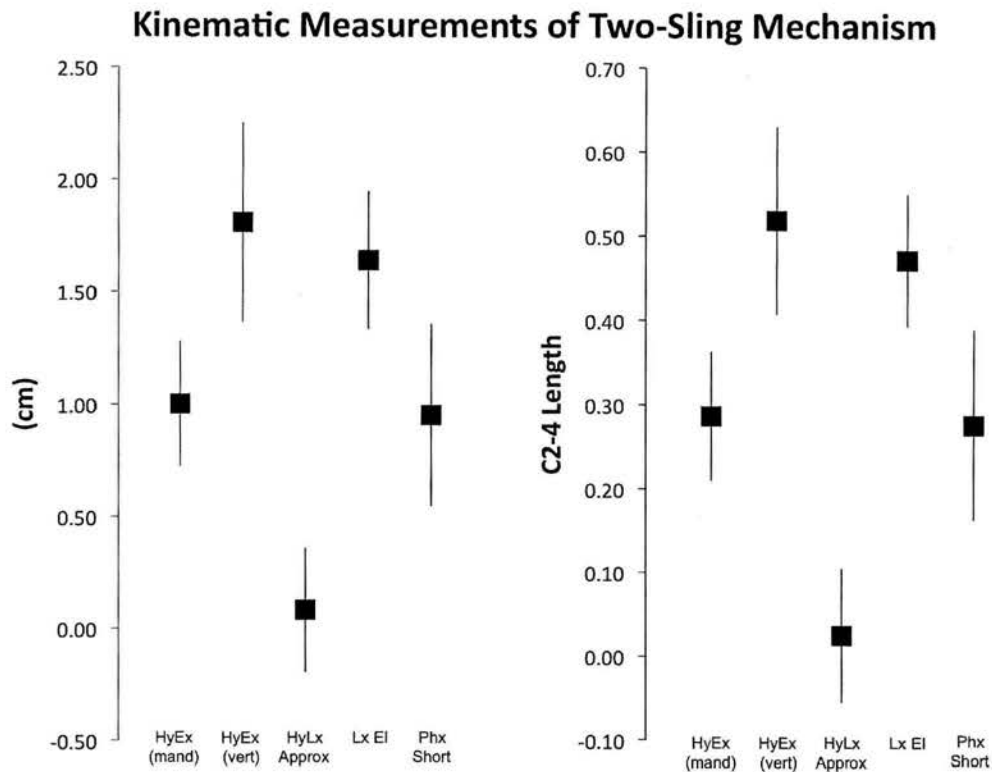

Fig. 59. Means and 95% confidence intervals of kinematic measurements representing actions of the anterior and posterior muscular slings are plotted (n=11). The graph on the left is scaled by centimeters whereas the graph on the right is scaled by C2-4 length to adjust for between subject size differences. Kinematic measurements potentially representing the action of the anterior muscular sling: HyEx (mand)= hyoid excursion measured against the axis of the mandible or HyEx (vert)=hyoid excursion measured against the axis of the vertebrae. Kinematic measurements potentially representing the action of the posterior muscular sling: LxEl= Posterior thyroid approximation to the cranial base, Phx short= pharyngeal shortening representing the action of the palatopharyngeus. Hyolaryngeal approximation (HyLx Approx) could represent the action either of the thyrohyoid, or of the posterior muscular sling to shorten the distance between the hyoid and larynx (both in motion during swallowing). HyEx, LxEl, and Phx short measurements here indicate the function of the anterior and posterior sling muscles is to actively shorten.

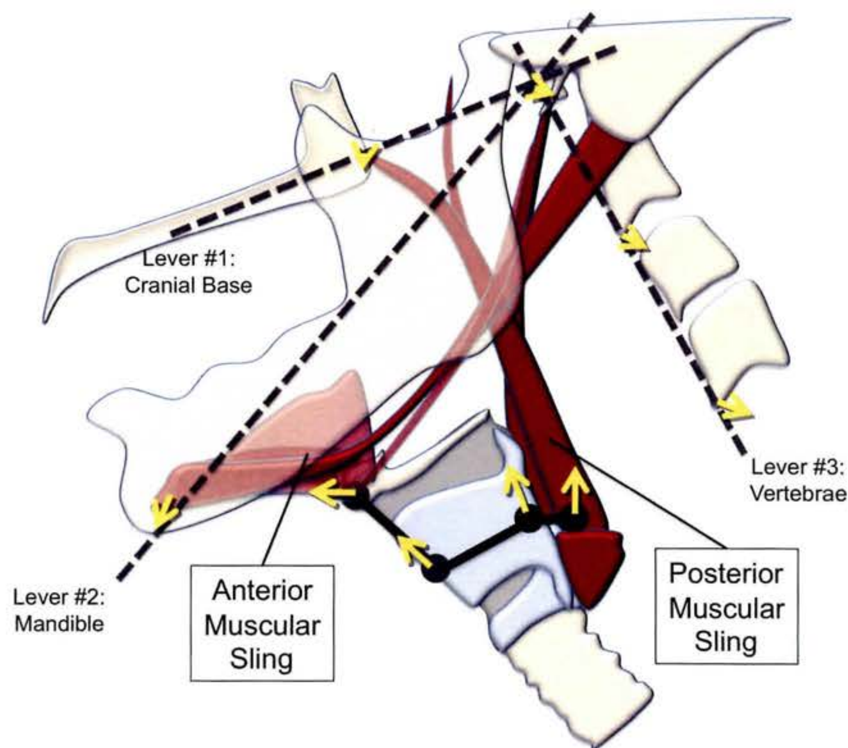

Fig. 60. Covariant shape changes indicated the action of both muscular slings. A diagram (in gray) of the two-sling mechanism, consisting of dashed lines and a solid line connecting large dots, is superimposed over an illustration of relevant anatomical structures. The three dashed lines represent skeletal levers including the cranial base (contiguous with the hard palate), mandible, and vertebrae mapped by 5 coordinates (mandible, posterior edge of hard palate, tubercle of the atlas, C2, C4). The solid gray line connecting large dots represents interconnected features of the hyolaryngeal complex mapped by four coordinates (hyoid, anterior larynx, posterior larynx, and the location of the upper esophageal sphincter). Each coordinate maps an element of the two-sling mechanism. Yellow arrows indicate the relative magnitude and direction of covariation of each coordinate during swallowing in a cohort of young healthy normal subjects.

Following these studies of young healthy adults in Chapters 4-6 we compared patients with dysphagia as a sequela of head and neck cancer (HNC) treatment with normal controls in order to investigate the clinical relevance of the two-sling mechanism. In this study we used the same coordinate methodology applied to videofluoroscopy to demonstrate reduced kinematics of the posterior sling muscles among post radiation HNC patients (Fig. 61). The test group was characterized by poor swallowing outcomes including penetration, aspiration, and residue, indicating that reduced posterior sling function is associated with poor swallowing outcomes. We also used shape change analysis to demonstrate the effect of HNC treatment on the two-sling mechanism as a system (Figs. 62,63). In this shape change analysis it is observed that in addition to reduced hyolaryngeal elevation, neck extension is increased and hyoid movement exaggerated, presumably as a compensatory mechanism of patients with dysphagia. In Chapter 8 the covariation of the two slings as functional modules is explored. It was found that covariation of the two slings is reduced among post treatment HNC patients regardless of age group indicating that the effect of pathology is to disrupt the functional integration of the two-sling mechanism (Fig. 64). Taken together these studies show that an understanding of the two-sling mechanism of hyolaryngeal elevation is clinically relevant. The findings of this thesis advance the field of dysphagia research in several ways and are discussed in the subsequent sections.

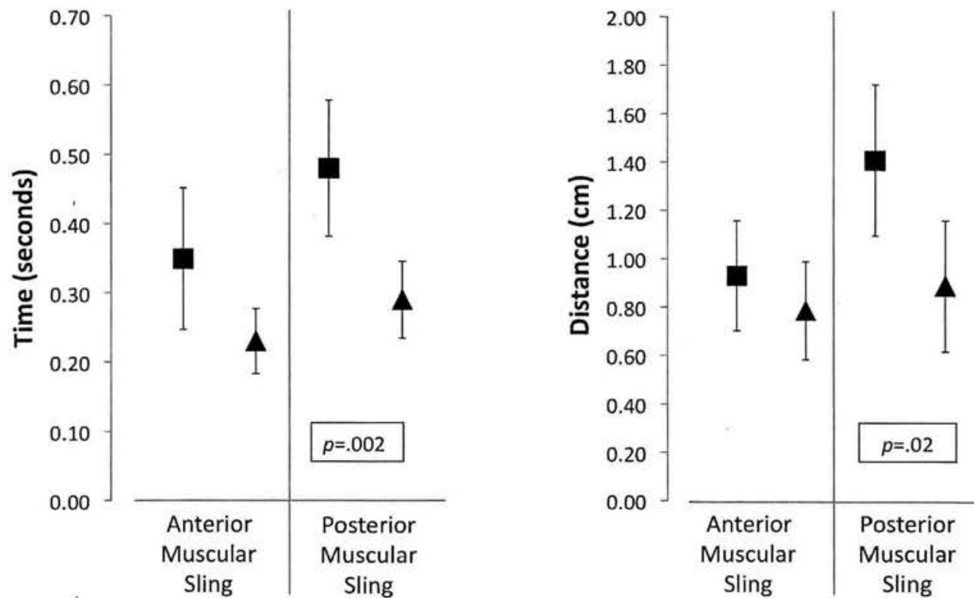

Fig. 61. Significant differences were found between control and test group (post-radiation head and neck cancer patients) for action of the posterior muscular sling based on both time and distance measurements of minimum to maximum hyolaryngeal movement in the pharyngeal phase of swallowing. No significant differences were observed in activity of the anterior muscular sling.

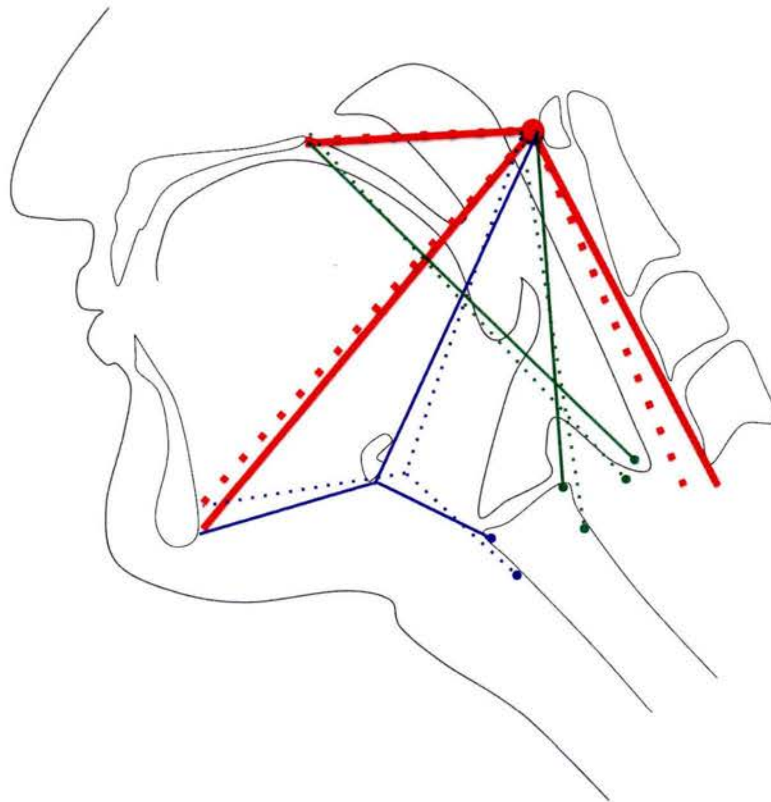

Fig. 62. Eigenvectors indicating shape changes in hyolaryngeal elevation of the control ('normal') group during swallowing. Dotted lines indicate mean shape at minimum hyolaryngeal excursion, and solid lines indicate the mean shape at maximum hyolaryngeal excursion. Thick red lines mark the position of the three skeletal levers (vertebrae, mandible, and cranial base). Darl blue lines mark the anterior muscular sling (suprahyoid muscles) and thyrohyoid muscle. Light green lines mark the posterior muscular sling (palatopharyngeus and stylopharyngeus).

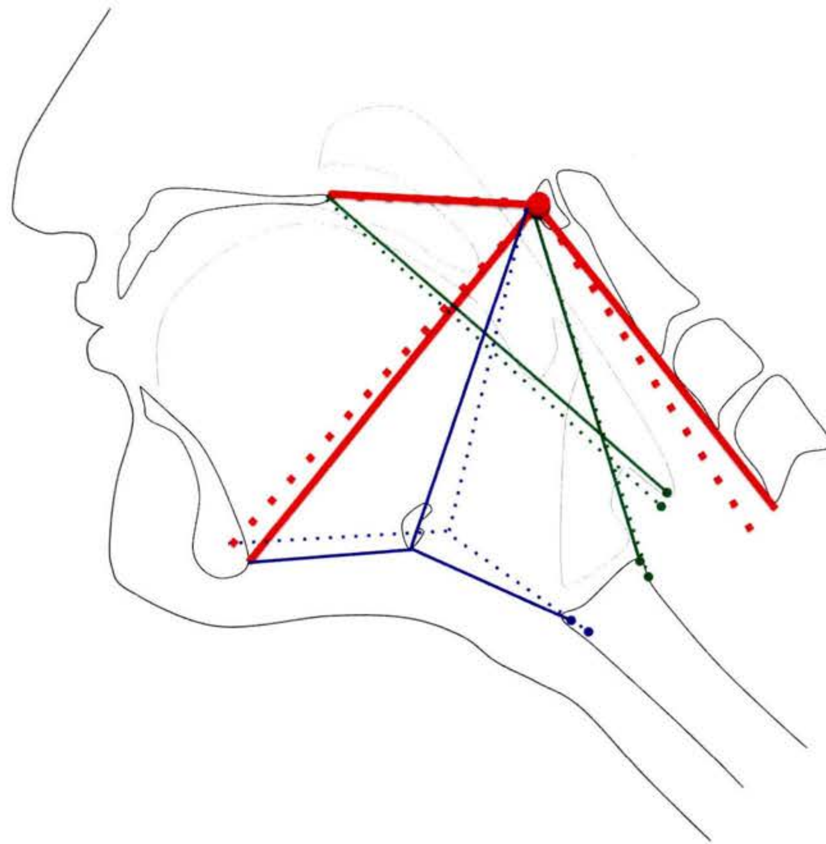

Fig. 63. Eigenvectors indicating shape changes in hyolaryngeal elevation of the post-treatment HNC group. Dotted lines indicate mean shape at minimum hyolaryngeal excursion, and solid lines indicate the mean shape at maximum hyolaryngeal excursion. Thick red lines mark the position of the three skeletal levers (vertebrae, mandible, and cranial base). Dark blue lines mark the anterior muscular sling (suprahyoid muscles) and thyrohyoid muscle. Light green lines mark the posterior muscular sling (palatopharyngeus and stylopharyngeus). Overall, changes are similar to but less marked than those in the control group. However, laryngeal elevation is notably reduced, extension of the head and neck is increased, and hyoid excursion is exaggerated versus controls.

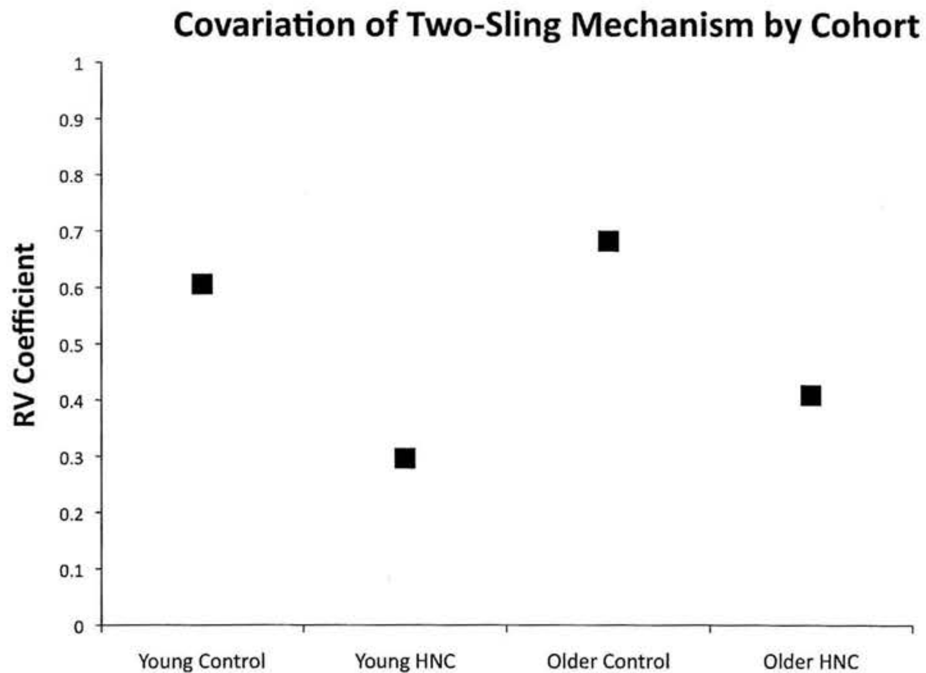

Fig. 64. Random variable (RV) coefficients document the covariation of the anterior and posterior muscular sling for each cohort (Young Control= normal swallowing <65 years old, Young HNC = post radiation head and neck cancer patients < 65 years old, Older Control = normal swallowing ≥ 65 years old, Older HNC = post radiation head and neck cancer patients ≥ 65 years old). These findings suggest that the function of the two slings is more integrated in normal subjects and becomes uncoupled as a result of HNC or HNC treatment.

Improved understanding of hyolaryngeal elevation

This thesis reconceptualizes hyolaryngeal elevation. The idea that the long pharyngeal muscles elevate the larynx and pharynx has been propounded in anatomy textbooks for many years (Lockhart et al., 1965). Without supporting evidence this has not found its way into current literature. It may be that since the long pharyngeal muscles are embedded deep within the head and neck and their function is difficult to measure, they were unintentionally omitted from direct investigation and, therefore, from consideration. Whatever the case, the current swallowing literature attributes hyolaryngeal elevation wholly to the suprahyoid muscles and the thyrohyoid muscle (Matsuo and Palmer, 2008). The studies in this thesis have shown that this is not the entire story, and that the long pharyngeal muscles also aid importantly in this function. Moreover, in our sample of post-radiation head and neck cancer patients with impaired swallowing, the function of the posterior sling muscles was significantly reduced, but that of the anterior sling muscles was not. This renewed focus on the role of the posterior sling musculature should help to stimulate research efforts and improve clinical interpretation of swallowing disorders.

Understanding of this mechanism allows for a more in-depth and nuanced interpretation of results reported in clinical studies. For example, the authors of a study of Wallenberg's syndrome (WS) assume that the submental muscles alone elevate the larynx (Aydogdu et al., 2001). WS is an insult to the lateral medulla that affects the nucleus ambiguus, which supplies the posterior muscular sling among other structures. This syndrome often results in patients losing the ability to feed orally. In their discussion, Aydogdu et al observed that the submental muscles had prolonged electromyographic activity in these patients, but could not explain this observation. A possible explanation to explore is that as two slings cooperate to effectively elevate the hyolaryngeal complex and the posterior sling is innervated by nucleus ambiguus, perhaps the anterior sling is attempting to compensate for a loss of posterior sling function.

Better understanding of the underlying two-sling mechanism could also lead to improved treatment for patients. Strength training of the swallowing muscles is commonly used in treatment for patients with dysphagia. These treatments are based on the idea that targeting muscle groups with task-specific exercises will improve swallowing outcomes. Investigators design these exercises based on their conceptual understanding of the underlying anatomy and its function. One study of the Mendelsohn maneuver (MM) found increased submental activity during the MM exercise (Wheeler-Hegland et al., 2008). Our findings in Chapter 4 confirmed that the MM was effective in targeting some of the submental muscles (mylohyoid and anterior digastric but not the geniohyoid) but was also very effective in targeting the posterior muscular sling. Because the studies described in this thesis have enriched our understanding of the swallowing mechanism, they have the potential to contribute to new and improved swallowing therapies.

Reconciliation of conflicting reports in the literature

Elucidating the underlying structure of the hyolaryngeal complex and its suspensory mechanism helps to untangle some conflicting reports in the literature. While the swallowing literature produced by speech language pathologists, gastroenterologists, and otolaryngologists supports the idea that the anterior muscular sling and thyrohyoid muscle are critical for hyolaryngeal elevation, a number of studies in the head and neck radiation oncology literature report that these muscles are not essential to swallowing function (Christianen et al., 2011; Eisbruch et al., 2004; Feng et al., 2007). Furthermore, these latter studies advance the idea that the pharyngeal wall muscles, including a portion of the posterior muscular sling, are the most relevant to swallowing function. While the problem may lie with inadequate methodology (discussed in Chapter 4) the apparent conflict reflects inadequate available knowledge of the subject. If two groups of investigators claim a different portion of a covariant mechanism is critical to hyolaryngeal elevation, they would not be entirely incorrect. In fact, the studies in this thesis offer a reconciliation of contradictory observations by demonstrating that hyolaryngeal elevation does involve both anterior and posterior muscular slings.

Insights into other swallowing functions

Results of the work described here lead to a reconsideration of hyolaryngeal approximation. This physiological function is currently attributed to the thyrohyoid muscle, which is a muscle that is intrinsic to the hyolaryngeal complex (Cook et al., 1989; Mepani et al., 2009). In Chapters 3 and 4 we demonstrated that the structure and function of the thyrohyoid is not sufficient to account for thyrohyoid approximation. We furnished evidence in Chapters 3-6 that the muscles of the posterior muscular sling elevate the larynx. We have argued specifically that the stylopharyngeus, which attaches directly to the posterior border of the thyroid cartilage, has a role in moving the thyroid cartilage toward the hyoid bone and thus achieves hyolaryngeal approximation. At a minimum, we can state that the evidence of thyrohyoid function in our studies does not agree with descriptions of thyrohyoid function in the literature. If the thyrohyoid is not recruited in swallowing then the data reported in Chapter 8 suggests that a shift from the posterior muscular sling as the primary elevator of the larynx to the anterior muscular sling is associated with aging.

Another swallowing event of interest is that of pharyngeal constriction. The very name of this function invokes the structure of the pharyngeal constrictor muscles. It is reasonable to assume that since the concentric contraction of the pharyngeal constrictor and the longitudinal pharyngeal muscles decreases the volume of the pharynx (according to Boyles law) both contribute to increased pharyngeal pressure (Leonard et al., 2011). However, it remains unknown which set of muscles is more important to pressure generation. One study showed that retention of bolus in the pharynx, usually associated with low pharyngeal pressure, was more highly associated with laryngeal elevation than pharyngeal pressure (Olsson et al., 1997). It has also been shown that posterior muscular sling is composed primarily of slow twitch muscle fibers, while the pharyngeal constrictors are primarily fast twitch (Mu and Sanders, 2007). This study demonstrated that the long pharyngeal muscles are active in hyolaryngeal elevation, but the set of

studies cited above indicate that they may also play an important role in sustaining pharyngeal pressure during swallowing, as do the constrictors.

Delineation of the dynamics associated with poor swallowing outcomes.

Chapters 7 and 8 explored the reduced elevation of the hyolaryngeal complex in a cohort of post-radiation head and neck cancer patients. In Chapter 7, kinematic data and shape changes showed that function of the posterior muscular sling is compromised in these patients, and this is associated with poor swallowing outcomes such as penetration-aspiration and residue. In Chapter 8 we demonstrate that the two-sling mechanism was more integrated functionally in the control group than in the post-radiation test group, regardless of age. Shape change and functional integration of the two slings provides additional insight into disordered swallowing.

New adaptations of existing methods will be useful for future research

In this thesis methods used in other studies were newly adapted to investigate the structure and function of the two- sling mechanism. Cadaveric studies have previously been performed to understand the underlying structures for particular functions (van Eijden et al., 1997). Muscle functional MRI has been used to study mastication (Yamaguchi et al., 2011). The kinematics of swallowing have been thoroughly defined (Leonard et al., 2000). Morphometric analysis is commonly used to evaluate shape changes between species (Klingenberg, 2010). Each method was adapted in this thesis to address our questions. Of these methods, the development of a system of nine anatomical landmark coordinates to map the two-sling mechanism in videofluoroscopy may have the greatest potential application to future dysphagia research and assessment. These coordinates can be used to calculate kinematic and shape change analysis and, by extension, address a wide variety of questions.

A major limitation of anatomical landmarking as used here, and with all videofluoroscopic measurement, is that three-dimensional structure is viewed and

interpreted in two dimensions. Useful future research would be an investigation of whether three dimensional kinematics correlate with landmarks that are currently measured in two dimensions. A research group in Japan uses 320-detector-row multi-slice computed tomography to track kinematics of swallowing in three dimensions (Inamoto et al., 2011). Future plans include using MorphoJ software, which can analyze coordinates in two and three dimensions, to evaluate the degree to which covariant shape change in three dimensions is mirrored in two dimensions.

For now, videofluoroscopy continues to be the gold standard of swallowing assessment. The landmarking data method to track the two-sling mechanism of hyolaryngeal elevation described in this thesis provides many variables of interest. Studies that combine these measures with other measurements including pharyngeal constriction ratio, distention of the upper esophageal sphincter, and timing variables would enable researchers to assess structure to function relationships underlying poor swallowing outcomes (penetration-aspiration or residue). These methods can also be used to define patient populations or test various therapies to improve swallowing.

Videofluoroscopy coupled with high-resolution manometry provides another opportunity to assess structure and function. A high-resolution manometry probe contains multidirectional sensors every 2 millimeters, enabling researchers to collect pressure data at any given time point throughout the length of the hypopharynx. Analysis of such physiological information in the context of changing structure as seen by fluoroscopy would provide more insight into swallowing and swallowing dysfunction.

Finally, coupling the methods from Chapter 4 and 5 provides a potentially useful research paradigm to further study the ability of swallowing exercises to target various muscles. Dynamic MRI (Chapter 5) can take place during the test conditions of swallowing or swallowing exercise tasks and mfMRI (Chapter 4) will measure the physiologic response

of muscle to those tasks. Therefore coupling these two methods in two planes provides an investigator with both functional and structural data in the same subject.

Limitations in scope

This study clearly demonstrates that reduced hyolaryngeal elevation is associated with increased penetration, aspiration, and residue. These poor swallowing outcomes are the consequence of an unprotected airway and insufficient bolus clearance (thought to be related to reduced opening of the upper esophageal sphincter). Present findings do not prove that hyolaryngeal elevation provides airway protection and greater opening of the upper esophageal sphincter.

Airway protection results from a combination of anterosuperior relocation of the hyolaryngeal complex, base of tongue covering the larynx, anterior movement of the arytenoids contacting the epiglottis and sealing off the laryngeal vestibule, and vocal fold adduction. In addition to the retrusion of the base of tongue, it is likely that hyolaryngeal elevation contributes to anterior arytenoid movement and epiglottic retroflexion (Van Daele et al., 1995). However, the evidence contained in this thesis does not prove that hyolaryngeal elevation alone protects the airway. More study is needed to investigate the interplay and importance of these various structures to airway protection.

We have reiterated throughout this work that the upper esophageal sphincter is a physiological construct created by the tonic contraction of the cricopharyngeus and the distal portion of the thyropharyngeus (Sivarao and Goyal, 2000). In Chapter 7 the distension of the UES was not measured as an outcome variable of hyolaryngeal elevation because of the stricture associated with radiation treatment. It is reasonable to presume that the anterior and posterior slings together stretch open a relaxed UES. Anterior traction provided by the anterior muscular sling has been shown to open the UES on an animal model (Asoh and Goyal, 1978). The posterior sling likely provides superolateral traction on the larynx and lateral pharyngeal wall to engulf an ingested

bolus. There have been cases of patients with a total glossectomy who can still swallow a syringe-fed bolus if they can elevate the hyolaryngeal complex (McConnel and O'Connor, 1994). The role of hyolaryngeal elevation in opening the upper esophageal sphincter, especially the role of the posterior muscular sling, presents an opportunity for future study.

Summary

In sum, this thesis found structural, functional, and clinical evidence supporting a two-sling mechanism for hyolaryngeal elevation. Although no new structures or functions were discovered, the coupling of structure and function was established in a new way, thereby providing insight into a critical function of swallowing that is associated with airway protection and effective bolus transport through the hypopharynx. By developing methods with a bias to quantify the functional capabilities of underlying structure, we provide the opportunity to develop future studies that will increase investigators' and clinicians' understanding of swallowing function and dysfunction. These methods can now be applied to many research questions that should improve swallowing ability for patients with dysphagia.

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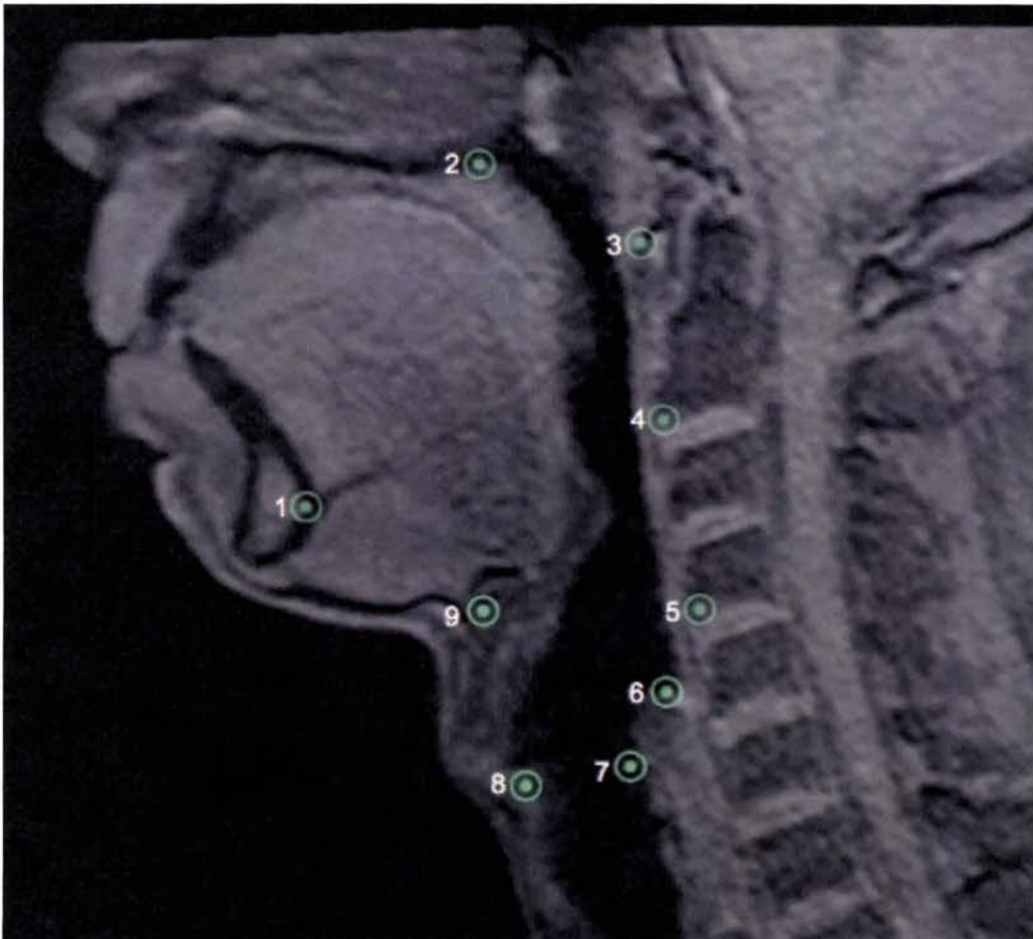
APPENDIX

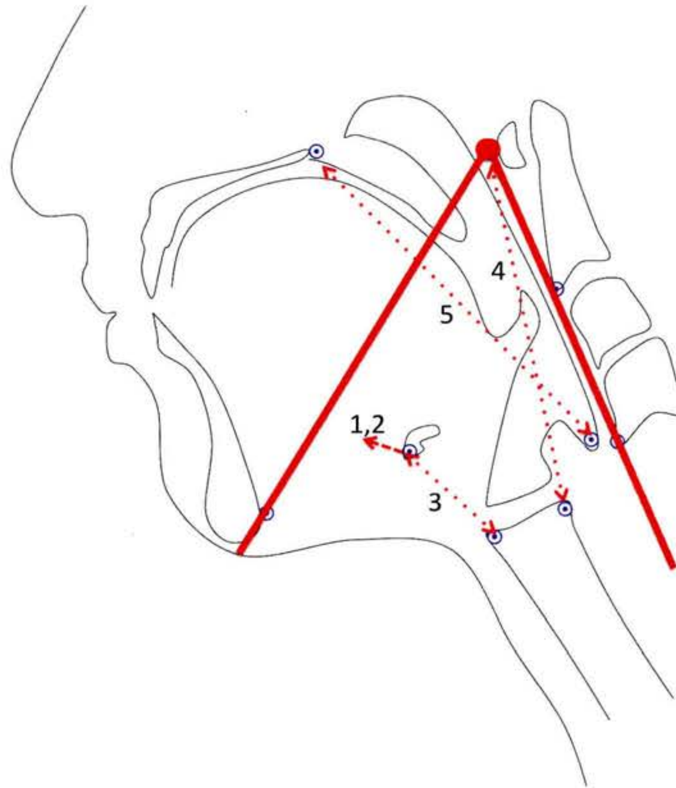
Methods used in chapter 5 and 7 to transform coordinates into kinematic measurements using trigonometry.

Coordinates: X,Y coordinates are collected from each frame as pictured below.



1=genial tubercles of the mandible, 2=posterior edge of the hard palate, 3=anterior tubercle of C1, 4=anterior inferior edge of C2, 5=anterior inferior edge of C4, 6=inferior air column of hypopharynx proximal to the upper esophageal sphincter, 7=posterior commissure of the vocal folds, 8=anterior commissure of the vocal folds, 9=anterior inferior edge of the hyoid.

Measurements:



Measurements used in chapter 5 include calculated distance measurements of: (1) Hyoid excursion with vertebrae as a reference (Anterior Muscular Sling); (2) Hyoid excursion with mandible as a reference (Anterior Muscular Sling); (3) hyolaryngeal approximation (thyrohyoid); (4) Posterior laryngeal approximation to cranial base (Posterior Muscular Sling); and (5) Pharyngeal shortening (Posterior Muscular Sling). Each measurement was reported in centimeters and against a subject specific anatomical scalar of C2-4 length. Measurements used in chapter 7 include measurement (2) and (4).

Table of measurements: what muscles actions measurements represent and a brief description of the measurement calculation.

Kinematic Measurement	Underlying muscle represented	Brief Description
Hyoid excursion with vertebrae as a reference	Suprahyoid muscles	This is a measurement of the movement of coordinate #9 in maximum and minimum hyolaryngeal elevation (min&max HLE) using the vertebrae as an axis of reference represented by a line between coordinate #3-#5.
Hyoid excursion with mandible as a reference	Suprahyoid muscles and likely deep neck muscles that flex and extend the head	This is a measurement of the movement of coordinate #9 in min&max HLE using the vertebrae as an axis of reference represented by a line between coordinate #1-#3.
hyolaryngeal approximation	Primarily the thyrohyoid	This measurement is the difference of the distance measurement from coordinate #8 to coordinate #9 at min&max HLE
Posterior thyroid approximation to cranial base	Stylopharyngeus	This measurement is the difference of the distance measurement from coordinate #3 to coordinate #7 at min&max HLE
Pharyngeal shortening	Palatopharyngeus	This measurement is the difference of the distance measurement from coordinate #2 to coordinate #6 at min&max HLE

Detailed instructions for calculating each kinematic measurement

In the following instructions min=minimum, max=maximum, and HLE=hyolaryngeal elevation, EQ#1=Pythagoras Theorem, and EQ#2 is the law of cosines solved for determining the angle of interest as described below.

EQ#1 $$a = \sqrt{(x_2 - x_3)^2 + (y_2 - y_3)^2}$$

EQ#2 $$B = \cos^{-1}\left(\frac{a^2 + c^2 - b^2}{2ac}\right)$$

1. Hyoid excursion with vertebrae as a reference: Distance measurements between coordinates #9, 3, and 5 are each calculated using EQ#1. Once these are calculated, they can be used as line measurements in EQ #2 to calculate angle #9-#3-#5. After this angle is measured, sine and cosine functions can be used to determine the anterior and superior distance of the hyoid respectively against the axis of line #3-#5 at representing min HLE. Next, this procedure is repeated in the image representing max HLE. The anterior and superior distance in the minimum frame is subtracted from the maximum. Finally, the anterior and superior excursion differences are placed in Pythagoras' theorem and the resulting hypotenuse is reported as the overall excursion of the hyoid bone. Care must be given to note any negative values of superior and anterior movement since the indication of direction (negative= lengthening and positive=shortening) in erased this final step.
2. Hyoid excursion with mandible as a reference: Distance measurements between coordinates #1, 3, and 9 are each calculated using EQ#1. Once these are calculated, they can be used as line measurements in EQ #2 to calculate angle #3-#1-#9. After this angle is measured, sine functions can be used with line #1-#9 as the hypotenuse to determine the distance of the hyoid from line #1-#3 in image representing min HLE. Once this is done, it must be repeated in the image representing max HLE. The distance of the hyoid from the axis represented by line #1-#3 in the maximum HLE frame if subtracted from the distance of the

hyoid from the axis represented by line #1-#3 in the minimum HLE frame. The result is reported as the overall excursion of the hyoid bone.

3. Hyolaryngeal approximation Distance measurements between Coordinates #8-#9 are each calculated using EQ#1 at min&max HLE. The difference is reported.
4. Hyolaryngeal approximation: Distance measurements between Coordinates #8-#9 are calculated using EQ#1 at min&max HLE. The difference is reported.
5. Posterior thyroid approximation to cranial: Distance measurements between Coordinates #3-#7 are calculated using EQ#1 at min&max HLE. The difference is reported.
6. Pharyngeal shortening: (Posterior Muscular Sling). Distance measurements between Coordinates #2-#6 are calculated using EQ#1 at min&max HLE. The difference is reported.

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