ORIGINAL ARTICLE



Electromyography of Swallowing with Fine Wire Intramuscular Electrodes in Healthy Human: Amplitude Difference of Selected Hyoid Muscles

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Abstract Few studies have examined the intensity of muscle activity during swallowing in healthy humans. We examined selected hyoid muscles using fine wire intramuscular electromyography (EMG) during swallowing of four food consistencies. Thirteen healthy adults were studied using videofluorography and EMG of the anterior belly of digastric (ABD), geniohyoid (GH), sternohyoid (SH), and masseter (MA; surface electrodes) while ingesting thin liquid (three trials) and solid food of three consistencies (banana, tofu, and cookie, three trials each). After rectification, integration, and normalization, peak EMG amplitudes for each muscle in each trial were measured. Hyoid displacements were measured in two dimensions. Data were analyzed using repeated measures ANOVA with Bonferroni correction. GH had the highest adjusted amplitude for both solids and liquid. For MA and ABD, amplitude was highest with triturated cookie. For ABD, amplitude was lowest with liquid. There were no significant food consistency effects for GH or SH. Hyoid displacements were greatest for cookie and the lowest for

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liquid. EMG amplitude varied with initial food consistency. The high peak EMG amplitude of GH is consistent with its essential role in opening the upper esophageal sphincter. High MA amplitude with hard solid foods is likely due to the higher tongue-palate pressure with triturated solids. The higher ABD amplitude with solid food is associated with greater hyoid displacement. These findings support the existence of a central pattern generator that modifies the level of muscle activity during pharyngeal swallowing in response to input from mechanoreceptors in the oral cavity.

Keywords Deglutition · Electromyography · Hyoid bone · Physiology · Rheology · Food

Introduction

During swallowing, movement of the hyoid bone, tongue, larynx, and other neck tissues is executed by a tightly coordinated group of muscles [1]. Several tools have been used by researchers and clinicians to analyze swallowing behaviors including videofluorography (VF), videoendoscopy, and surface electromyography (EMG). However, none of these tools can determine the strength of activation of individual small muscles during swallowing. It is known, from animal data and human studies, that the suprahyoid muscles are activated early in swallowing [1-4]. Suprahyoid muscle surface EMG has demonstrated activations of the suprahyoid muscle group, including the mylohyoid, anterior belly of digastric (ABD), and geniohyoid (GH) muscles [5]. Therefore, studies using intramuscular electrodes are necessary to capture individual muscle activity as several small muscles are activated sequentially in a small area during swallowing. Compared to needle electrodes, fine wire electrodes are convenient for insertion in small muscles, allow painless muscle movement for the participant [6–10], and provide stable measurement without significant electrode displacement within the muscles [7, 8, 11, 12]. Previous research using fine wire electrodes has shown that muscle activation during swallowing follows a typical sequence and suggested that activation timing is affected by food consistency [8].

It is well recognized that swallowing patterns change with food consistency. Duration of food transit through the oropharynx is longer when swallowing high-viscosity foods [13]. Other studies have demonstrated higher activation of the suprahyoid muscle group when swallowing viscous food [14]. However, no study has shown the amplitude differences among individual muscles by food consistency.

Anterior displacement of the hyoid bone leads to upper esophageal sphincter opening and contributes to superior displacement of the larynx [1]. The hyoid is connected to several muscles including GH, digastric (both anterior and posterior belly), mylohyoid, thyrohyoid, sternohyoid (SH). Several studies have evaluated hyoid displacement with different food consistencies during swallowing [15, 16], but, to our knowledge, no study has reported hyoid displacement together with individual muscle activation.

The primary aim of the current study was to analyze EMG signals during swallowing to determine the degree of activation in selected hyoid muscles. Based on previous kinematic studies, we hypothesized that the selected suprahyoid muscles would have distinct activation patterns on EMG and that activation amplitude of each muscle would differ by food consistency. Using hooked wire intramuscular electrodes to collect EMG data, we analyzed muscle activation during swallowing several food consistencies. We also evaluated the hyoid displacement.

Materials and Methods

Materials and methods describing EMG have been described previously [8].

Participants

Fourteen healthy participants [age 22 ± 4 (mean \pm SD) years, nine women, five men] were recruited for this study; none had any history of major medical problem, dysphagia, or abnormal dental occlusion. Participants were screened for swallowing dysfunction before enrollment by drinking 5 ml of liquid barium during VF (lateral and anterior-posterior views). The protocol was approved by the Institutional Review Board of our institution. Each participant provided verbal and written informed consent.

Electrode Placement

Hooked wire intramuscular electrodes were inserted into the right SH, ABD, and GH. The overlying skin was anesthetized using 1 % Lidocaine with Epinephrine. Then, a monopolar needle electrode was inserted in the right side of the neck to identify the location of the muscle. Muscle contraction was evoked by electrical stimulation to establish needle position. The monopolar electrode was removed and the hooked wire electrodes (two 40-gauge Tefloncoated steel wires in a 27-gauge 12.5 mm hypodermic needles) were inserted at the same location and depth. After the hypodermic needle was removed, the muscle was stimulated again through the wire electrodes to verify that the contraction pattern matched that of the muscle of interest. Participants reported minor local discomfort due to electrode placement, but none felt that the study should be stopped or that it disturbed chewing or swallowing. If a wire electrode was accidentally moved or disengaged during the experiment, it was not replaced; subsequent data from that electrode were excluded from the analysis as there is regional variation of EMG activation [11, 12].

Surface EMG of the right masseter (MA) was recorded using disposable surface electrodes (Disposable Tab Electrodes, VIASYS Healthcare, Madison, WI, USA) after determining its position by palpation. All EMG recordings were inspected in real-time to exclude waveform changes indicative of the electrode slippage.

Data Collection

VF and EMG of GH, ABD, SH, and MA were recorded simultaneously as participants ingested various types of food with or without barium. VF was done using a 12-inch image intensifier with appropriate collimation while obtaining the image of the entire mouth and pharynx. Video output was recorded using a digital video tape recorder at 30 frames/s. A video-timer added a time signal to each frame of the video recording in 0.01 s increments. Small radiopaque lead disk markers, 4 mm in diameter and 0.5 mm thick, were cemented on the buccal surfaces of the left upper and lower canines and first molars using dental adhesive.

Participants were seated comfortably and were instructed to minimize head movements during recording. Three trials were performed for each of the following consistencies: (1) drinking 10 ml of thin liquid, and eating 6 g each of (2) banana, (3) firm tofu, and (4) hard cookie (Pure Butter Shortbread[®], Walkers Shortbread Inc.). These initial food consistencies and bolus sizes were selected on the basis of extensive prior studies in our laboratory and others [8, 17–19]. They present a range of rheological characteristics, thus presenting different demands on the system for food reduction, bolus formation and transport, and swallowing. In particular, the liquid has low viscosity; the banana is soft and highly adhesive; the tofu is soft with low adhesiveness; and the cookie is quite hard [19]. For the first trial of each food consistency, barium contrast was added to evaluate eating behavior, while subsequent trials were done without barium necessary because barium obscures the markers. Because the disk markers fell off frequently during cookie ingestion, it was always done at the end of each session. For liquid trials, the participants were asked to swallow on command. For solid food trials, participants placed the food in the mouth and were instructed to chew and swallow in their usual manner. Recording began immediately before ingestion and continued until the mouth was completely cleared. Thirteen of 14 participants accomplished the full protocol. As the remaining participant ate more slowly, the recording was terminated at 5 min as per the radiation safety protocol.

Data Analysis

EMG activation was monitored and recorded to digital audio tape with analog to digital conversion at sampling rate of 1.5 or 3 kHz on a LX-10 (TEAC, Tama, Tokyo, Japan). We used a program, SEQUENCER (written by David Hertweck), which performs three processes: (1) rectify and integrate using constant of 10 ms, (2) delete data with amplitude below 20 % of maximal amplitude to reject the background activity and to reduce noise, and (3) calculate moving averages for each 1/30 s to obtain numeric amplitude data [20, 21]. The moving average is a function of both motor unit amplitude and discharge frequency. The maximum amplitude of each muscle for every participant was determined over all trials. To compare amplitudes among trials and participants, we calculated normalized amplitudes by dividing the averaged numeric amplitude for each 1/30 s by the maximum amplitude of each muscle in each participant.

We added temporal axes on the EMG data using the onset of GH activity as a reference time (0 s) of EMG because it is known that GH activates more consistently during swallowing [22, 23]. GH activation was also highly stable during swallowing in our study. We selected and analyzed the peak of adjusted amplitudes for each muscle from -1 to 1 s relative to GH onset.

VF recordings were imported to a desktop computer with digital image processing software (ImageJ, U.S. National Institutes of Health, Bethesda, MD, USA) for analysis. The recordings were reviewed in slow motion and analyzed frame-by-frame using the stop-motion capabilities on the software.

The recording of two lattice gauge grids, with each 0.5 and 1 inch squares, were used to indicate true distances in

the image. The position of several anatomical reference points were transformed to Cartesian (X and Y) coordinates; the two markers on the upper canine and first molars on the left side and the hyoid bone. A frame of reference was set using a line between the upper canine and the upper molar, representing the upper occlusal plane, as X axis. A perpendicular line to the X axis at the upper canine was used for Y axis. We used the antero-superior corner of the hyoid bone and measured displacement in X (horizontal) and Y (vertical) co-ordinates (Fig. 1).

We chose the first swallow of each trial and defined as our reference times (0 s) of VF the start of rapid anterior hyoid movement. This event was chosen because it consistently differentiates swallowing from mastication and other motions [1]. We selected -1 to 1 s data as the swallowing sequence of interest and calculated maximum X and Y displacements of the hyoid.

Statistical Analysis

To determine the factors affecting peak adjusted amplitudes, we used repeated measures analysis of variance (ANOVA) and compared each variable using Bonferroni post hoc test. P < 0.05 was considered significant. Analysis was performed using Stata version 11 (Stata Corporation, College Station, TX, USA).



Fig. 1 An example of the reference line in videofluorography. Four markers were attached to the left teeth, upper and lower canines, and first molars. We used a reference line using a line between the upper canine and the upper first molar (*circled*), which represented the upper occulusal plane, as *X* axis (*a*). A perpendicular line to the *X* axis at the upper canine was used for *Y* axis (*b*). We measured a displacement of the antero-superior corner of the hyoid bone (*c*) in *X* (*horizontal*) and *Y* (*vertical*) co-ordinates

Results

One participant was excluded due to anterior dislocation of the jaw during opening seen on VF in lateral view. Thus, 13 participants were included in the study. For each participant, we recorded three trials of each food (12 trials) except for one participant who had five trials of banana and one who had two cookie trials. A total of 157 sequences were obtained including (1) 39 sequences of liquid swallow, (2) 41 sequences of banana, (3) 39 sequences of tofu, and (4) 38 sequences of cookie. SH data of one participant were excluded due to poor quality, reducing the number of SH sequences analyzed to 146. No wire electrode disengagement occurred during the experiment, thus, whole recordings were taken without replacing the electrodes.

Adjusted Amplitude

For all food consistencies, GH had higher peak adjusted amplitude compared to other muscles (Table 1). Muscle had strong effect on peak adjusted amplitude (F = 57.54, P < 0.01; Table 2). A significant difference in muscle activation amplitude was found; GH was the highest followed by ABD.

After separating the sequences by muscle and food consistency, different patterns emerged. With cookie, MA had a significantly higher peak amplitude compared with other foods (F = 21.66, P < 0.01; Fig. 2; Table 3). ABD had a significantly lower peak amplitude with liquid than solids (F = 16.06, P < 0.01). On repeated measures ANOVA, trial order had a significant influence on ABD peak amplitude; while ingesting the same food consistency, the first trial showed higher peak amplitude than the last trial (F = 5.16, P < 0.01). The same pattern for trial order was seen in MA and GH (MA: F = 2.67, P = 0.03, GH: F = 2.68, P = 0.03). No statistically significant differences in peak amplitude were found for the GH and SH by food consistencies (GH: F = 0.31, P = 0.82, SH: F = 1.95, P = 0.12).

Hyoid Displacement

Hyoid displacement was the smallest during liquid swallows, the middle during banana and tofu swallows, and the greatest during cookie swallows (Table 4). Repeated measures ANOVA showed that consistency had a large effect on both horizontal and vertical hyoid displacement; maximal hyoid displacement was largest during cookie swallows and smallest during liquid swallows (Hyoid X: F = 151.7, P < 0.01, Hyoid Y: F = 116.7, P < 0.01; Fig. 3; Table 5).

Discussion

Maximal EMG amplitudes during swallowing varied with initial food consistencies, and the effects of consistency varied among muscles. Significant differences among food consistencies were found in the MA and ABD, and consistently high amplitude was found in the GH for all consistencies, though EMG recordings during swallowing are known to have substantial variability [11, 24]. These findings support a hypothesis that afferents from mechanoreceptors in the oral cavity project to the central pattern generator for pharyngeal swallowing [25, 26], which in turn modulates muscle activity during swallowing. One advantage of intramuscular EMG over other form of motion analysis is that myoelectric activity in skeletal muscle directly reflects neural activity. Thus, intramuscular EMG recordings reflect peripheral neural activity controlled by the central nervous system; contraction of swallowing muscles requires activation by lower motor neurons whose cell bodies are in brainstem somatic motor nuclei.

Higher-viscosity food is associated with larger hyoid displacement during bolus propulsion through the pharynx and upper esophageal sphincter. It is known that swallowing triturated solid food results in greater superior [15, 16, 27] and anterior displacement [16, 27] of the hyoid bone. Our study is consistent with previous reports in

 Table 1
 Peak adjusted amplitude for each muscle during swallowing relative to maximum amplitudes of each participant [median (interquartile range) (%)]

	MA	GH	ABD	SH
Liquid $(n = 39)$	9.5 (3.7–13.1)	44.2 (22.1–77.6)	17.6 (9.4–32.9)	11.8 (3.4–27.7)
Banana $(n = 41)$	11.4 (4.1–22.9)	56.1 (3.5–71.2)	33.9 (17.9–44.1)	18.3 (8.9–37.0)
Tofu $(n = 39)$	14.9 (7.6–24.0)	56.8 (28.6-73.7)	38.5 (20.1-58.6)	25.7 (9.8-32.3)
Cookie $(n = 38)$	37.1 (15.0–71.4)	52.4 (37.6–64.2)	51.1 (27.3–65.5)	22.4 (15.3–39.0)

All amplitude has been adjusted by maximum amplitudes of each participant

GH geniohyoid, MA masseter, ABD anterior belly of digastric, SH sternohyoid

	F	Р	Post hoc test
Total model	13.79	<0.01	
Muscle effect	57.54	< 0.01	$MA = SH < ABD < GH^*$
Food consistency effect	13.04	< 0.01	
Trial effect	4.93	<0.01	

Table 2 Repeated measures ANOVA for peak adjusted amplitude of each muscle and food consistency

Post hoc test performed with Bonferroni test

F F value, P P value, GH geniohyoid, MA masseter, ABD anterior belly of digastric, SH sternohyoid

* P < 0.05 for post hoc test of each pairs of muscles, except no significant difference between MA and SH

Fig. 2 Graph of peak adjusted amplitude among muscles for each food consistencies. The adjusted amplitude was calculated by dividing the numeric amplitude by the maximum amplitude of each muscle for every participant. For masseter (MA), cookie had higher peak than other foods. For geniohyoid (GH) and sternohyoid (SH), there were no significant differences among food consistencies. For anterior belly of digastric (ABD), liquid had lower peak than solid foods. Significant difference from peak adjusted amplitudes at *P < 0.05



which both anterior and superior hyoid displacements were larger with solids than liquids. Hwang et al. [19] reported that bolus viscosity after mastication was higher for hard cookie than for banana or tofu, and viscosities of solid food they reported were higher than that of liquid. Thus, the initial bolus consistency before mastication affects the consistency of that bolus after mastication. The changes in muscle activity in swallowing could reflect a feedback mechanism wherein afferents from the oral cavity and pharynx alter the swallow as it occurs. Alternatively, the change in swallowing could be produced by a feed-forward mechanism wherein the sensation from the oral cavity before swallowing has a direct effect on the motor activity in the subsequent swallow. We cannot exclude the possibility that both mechanisms are at work.

Peak amplitude differences by food consistency in MA and ABD suggest that the strength of muscle contraction is modulated to optimize the swallow based on the food consistency. Greater activation of the MA when swallowing triturated solid food could reflect the need for higher intra-oral pressure when swallowing higher-viscosity foods. It has been reported that liquids or triturated solid foods of higher viscosity require higher intra-oral pressure for bolus transport [14, 28]. Greater activation of the ABD with hard solid food (cookie) is likely related to the greater hyoid displacement discussed above. The ABD, which attaches the hyoid to the mandible, moves the hyoid upward and forward in concert with GH. Several studies [5, 8, 22] have shown that the GH and ABD are activated almost simultaneously during swallowing, though effects of food consistency differed between these two muscles. The consistently high level of GH EMG activity across food consistencies suggests that GH is a key muscle in the sequential muscle activation produced by the brainstem central pattern generator during swallowing. This is consistent with its hypothesized role in producing antero-superior displacement of the hyolaryngeal complex and opening of the upper esophageal sphincter. The ABD is activated at the same time as the GH, but, unlike the GH, its peak amplitude differs with food consistency. Its higher activity with cookie, taken together with its pattern of anatomical attachments, suggests that it produces the

	F	Р	Post hoc test
GH model	10.97	<0.01	
Food consistency effect	0.31	0.82	$L = B = T = C^{*^a}$
Trial effect	2.68	0.03	
MA model	5.79	<0.01	
Food consistency effect	21.66	<0.01	$L = B = T < C^{*^{\mathrm{b}}}$
Trial effect	2.67	0.03	
ABD model	11.03	<0.01	
Food consistency effect	16.06	<0.01	$L < B = T = C^{*^{c}}$
Trial effect	5.16	<0.01	Trial $1 > \text{Trial } 3^{*d}$
SH model	18.22	<0.01	
Food consistency effect	1.95	0.12	$L = B = T = C^{*a}$
Trial effect	0.22	0.92	

Table 3 Repeated measures ANOVA for peak adjusted amplitude of each muscle

Post hoc test performed with Bonferroni test

F F value, *P P* value, *GH* geniohyoid, *MA* masseter, *ABD* anterior belly of digastric, *SH* sternohyoid, *B* banana, *C* cookie, *L* liquid, *T* tofu ^{*a} There is no significant difference among food consistencies; ^{*b} P < 0.05 for post hoc test of each pairs of food consistencies that cookie has significantly higher activation than others; ^{*c} P < 0.05 that liquid has significantly lower activation than others; ^{*d} P < 0.05 for post hoc test of each pairs of trials that trial 1 has significant higher activation than trial 3

Table 4 Maximal hyoid displacement in X and Y axis [mean \pm s-tandard deviation (mm)]

		Maximal displacement
Hyoid X	Liquid $(n = 39)$	12.7 ± 2.9
	Banana $(n = 41)$	18.6 ± 3.3
	Tofu $(n = 39)$	19.4 ± 3.5
	Cookie $(n = 38)$	22.1 ± 4.2
Hyoid Y	Liquid $(n = 39)$	13.5 ± 4.4
	Banana $(n = 41)$	22.2 ± 3.3
	Tofu $(n = 39)$	22.4 ± 3.4
	Cookie $(n = 38)$	26.6 ± 4.4

greater displacement of the hyoid bone in swallowing triturated hard food. SH amplitude, however, did not vary significantly with food consistency, consistent with a prior report [29]. We infer that the hyoid's return phase is powered by traction of the larynx and trachea and does not require contraction of the infrahyoid musculature.

Habituation, attenuation of response over repeated trials, might be observed in the swallowing musculature. Habituation has been reported for the MA [30] and intercostal muscles [31] but not in the muscles of swallowing, per se. In the present study, repeated measures ANOVA revealed reduction in the ABD, MA, and GH EMG amplitudes over multiple trials. This could reflect habituation, though we cannot exclude the possibility that the position of the electrodes within each muscles changed over time with respect to the motor unit architecture.



Fig. 3 Graph of maximal displacement of hyoid among food consistency. Both horizontal and vertical displacements were the smallest for liquid and the largest for cookie. Significant difference from maximal hyoid displacements at *P < 0.05

		F	Р	Post hoc test
Hyoid X	Total Model	41.99	<0.01	
	Food consistency effect	151.7	< 0.01	$L < B = T < C^*$
	Trial effect	0.22	0.92	
Hyoid Y	Total Model	23.14	< 0.01	
	Food consistency effect	116.7	< 0.01	$L < B = T < C^*$
	Trial effect	0.19	0.94	

Table 5 Repeated measures ANOVA for maximal displacement of the hyoid of each muscle and each trial

Post hoc test performed with Bonferroni test

F F value, P P value, L liquid, B banana, T tofu, C cookie

* P < 0.05 for post hoc test that liquid has significantly lower hyoid displacements for liquid and higher displacements for cookie in both X and Y axes

Conclusion

Maximum EMG amplitudes during swallowing in selected hyoid muscles vary with initial food consistency. These findings support the notion that afferent signals from mechanoreceptors in the oral cavity project to the brainstem central pattern generator for swallowing and modify their motor output to the muscles of oral and pharyngeal swallowing. Further studies should explore in greater depth the effects of specific physical characteristics on the motor output to these muscles, and extend this approach to analysis of additional muscles involved in swallowing and related activities.

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Conflict of interest The authors report no conflict of interest.

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