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Description					

Use of barometric pressure and electromyography measurement techniques to elucidate the mechanisms by which bolus passes from the oral cavity to the oropharynx during swallowing

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<u>Highlights</u>

- 1. Swallowing timings were evaluated by two barometers in oral cavity and oropharynx.
- 2. Anterior and posterior intrinsic tongue EMGs were also recorded.
- 3. Timings of nasopharyngeal closure could be measured by the sensor in oral cavity.
- Durations of oral and pharyngeal stages of swallowing were measured as 0.4 and 0.6 sec.
- 5. The tongue begins the activity for swallowing before nasopharyngeal closure.

<u>Abstract</u>

Objectives:

The exact timings associated with swallowing are difficult to measure with the currently available methods. In this study, we aimed to elucidate the timings of action of the swallowing organs in the oral and pharyngeal stages of swallowing by recording the barometric pressure (BP) and tongue muscle activities.

Methods:

Dry and water swallows were studied in 10 adults using electromyography and small barometers. BPs were recorded during swallowing in the oral cavity (BP-o) and pharynx (BP-p), associated with muscle activities of the anterior (TA) and posterior (TP) parts of the tongue and of the suprahyoid (SHy) muscle. To analyze the temporal pattern of each activity, times of onset, cessation, and peak were measured.

Results:

Two characteristic waveforms were obtained. BP-o peaked immediately after onset and decreased to atmospheric pressure following a short plateau. However, BP-p gradually increased, reached a peak, and returned to the atmospheric pressure immediately before the end of BP-o. Since pressure increments indicated that the sensor was compressed in a closed space, onset and cessation of BP-p could correspond to the duration of nasopharyngeal closure. The onset of BP-p and the peak time of BP-o occurred in close succession. Thus, nasopharyngeal closure could be evaluated from BP-o. The sensor and EMG measured durations of oral and pharyngeal stages as 0.4 and 0.6 sec, respectively. TA activation began earlier than the TP. TA and TP peaks appeared before the BP-o peak, suggesting that the tongue begins the activity for swallowing before nasopharyngeal closure.

Conclusions:

This study revealed movements of swallowing organs in the two stages with high temporal resolution. BP-o detected the duration of nasopharyngeal closure.

Keywords

Swallowing, Electromyography, Barometric pressure, Tongue, Oropharynx,

Nasopharyngeal closure

Introduction

The human tongue participates in several oral motor functions including chewing, sucking, swallowing, respiration, and speech [1,2]. The process of swallowing has been described as a sequence of the following four consecutive stages: the preparatory, oral, pharyngeal, and esophageal stages. During the oral stage, the bolus is propelled from the oral cavity into the pharynx [3]. The use of videofluorography (VF) in a previous study had shown that the tongue plays a critical role in forming a food bolus and in transporting it from the oral cavity into the pharynx [4]. During food transportation oral structures, such as the lips, tongue, and soft palate change their shapes to squeeze the food bolus. Biplane VF has revealed the existence of an oropharyngeal propulsive chamber, which expels the food bolus into the hypopharynx, during swallowing [5]. Pushing the food bolus may be the tongue's main task, and a gradient of intraoral pressure may support bolus transport. Ono et al. has recorded tongue-to-palate pressure with multiple force sensors on the palate during swallowing. These have indicated that the order of tongue contact against each part of the hard palate and the duration and magnitude of tongue pressure are coordinated precisely during swallowing [6]. However, the discussed pressure was the contact force between the tongue and palate. Hence, understanding the mechanism by which the tongue generates propelling forces during the oral stage of swallowing is

necessary.

The role of barometric pressure (BP) changes on bolus transportation during oropharyngeal swallowing [7,8]. The oral cavity is composed of hard (e.g., hard palate, teeth, and jaws) and soft (e.g. tongue, soft palate, cheeks, floor of the mouth, and gingiva) parts, and is divided into small spaces, such as the inter-occlusal space, sub-palatal space, mid-pharyngeal space, and naso-epipharyngeal space. These structures change their size and/or number of spaces constantly during functioning [9]. These morphological changes may influence BP in the oral cavity (BP-o) and thus, manometry could be a tool for evaluating tongue function during swallowing. Santander et al. [7] found the occurrence of negative pressure during swallowing, which was dependent on the kind of food bolus applied and its consistency. Olthoff et al. [8] observed intraoral BP changes by a manometer along with visual observation of the organs using a magnetic resonance imaging (MRI) movie. A negative BP-o was reported to occur in association with laryngeal elevation during swallowing, along with submental muscle contraction. The authors concluded that the BP-o reduction during swallowing is not related to oral bolus transport, but supports laryngeal elevation via the palatal fixation of the tongue. Thus far, no study has proved the role of BP gradient on food bolus transportation during the oral stage of swallowing. Hence, a small sensor (5 x 3 x 2 mm) had been used to measure the positive and negative changes

in BP associated with swallowing [10,11]. Hasegawa et al. [11] assessed the swallowing mechanics in terms of BP changes in the oropharynx (BP-p), and found biphasic BP responses for the bolus swallow whereas, a single stage response for the dry swallow (DS). Jones et al [12] reported similar pattern. Based on the previous literature [10,11], we hypothesized that the peaks appearing in BP-o and BP-p during swallowing might be closely related, and this relationship could explain the mechanism by which the food bolus gets propelled into the oropharynx from the oral cavity.

Electromyography (EMG) recordings have been used for swallowing studies, since it is a noninvasive and inexpensive technique [13,14]. Submental EMGs may reflect the movement of floor-of-the-mouth muscles. Palmer et al. [2] measured the activity of submental EMG and the activities of five individual muscles during swallowing, and concluded that the primary contributions to the submental EMG were the mylohyoid, anterior belly of the digastric, and geniohyoid muscles, but not the genioglossus muscle (GG). Tsukada et al. [15] studied the temporal relationship between the suprahyoid muscle (SHy) and GG muscles using VF. They found that GG and SHy began their activities at almost the same time. At the beginning of the swallow, the tongue scoops the food bolus to a supralingual position, when the tongue tip reaches the posterior aspect of the maxillary incisors [3]. Thus, the onset of muscle

7

activity at the tongue tip may be the point at which the oral stage of swallowing starts. Until now, no study has reported the intrinsic tongue muscle activities in humans during swallowing.

Currently, VF [4] may evaluate the timings of the swallowing events; however, the time resolution of VF is low (approximately 30 ms). Therefore, temporal relationships should be confirmed with a BP sensor (3 ms of time resolution) and tongue EMG using simultaneous recordings. The purpose of this study was to elucidate the timings of the swallowing organs in the oral cavity and oropharynx by recording BP changes and tongue muscle activity, so that the precise relationship between the oral and pharyngeal stages of swallowing may be clarified.

Materials and Methods

The study was performed in accordance with the Helsinki Declaration II and was conducted with the approval of the Ethic Committee of Tokyo Dental College. All study participants received verbal and written information regarding the study and gave written consent to participate.

1. Subjects

Ten healthy volunteers (seven men and three women; mean age of 33.3 ± 12.4 years) with normal occlusion, normal swallow, and no oral and maxillofacial diseases were recruited from the staff and student population of the study institution. During the experiment, the subjects were seated in a relaxed upright position without head support.

2. Electrode and sensor settings

To elucidate the relative timings of swallow events, BP changes and EMG activities were recorded (Figure 1).

We used BP sensors (MPL115A1: Freescale Semiconductor, USA) that could measure absolute BP in a small space (sensor size: $5 \times 3 \times 2$ mm) with a 3 ms time resolution for BP measurements. This BP sensor was the same as used in the previous studies [10,11,16]. The BP-o was fixed to the center of ah-line at the oral appliance, which supported the wires of EMG for the tongue. The BP-p was introduced in the oropharynx, near the uvula via the nasal cavity with a guide wire. After visual confirmation of the sensor position (located near the lower part of the uvula) the guide wire was removed, and the cable of BP sensor was taped on the face. To protect this sensor from secretions, it was covered with thin and soft plastic covering. The signal from the BP sensor was decoded digitally with 3 ms intervals using the same micro-central processing unit (CPU) as for the EMG recording.

EMGs were obtained from the tongue and SHy muscles. Using surface electrodes, tongue muscle activity was recorded at two sites, on the dorsum of the tongue, i.e. at the anterior part of the tongue (TA) and the posterior part of the tongue (TP). Two silver plates (3 mm in diameter) covered with silicone rubber were glued symmetrically to the midline 2 cm apart from each other at the front part of tongue (about 1 cm posterior to the tongue tip) using a cyanoacrylate adhesive (Aron Alpha A "Sankyo", Daiichi Sankyo Inc, Japan), by the same techniques as was employed in a previous study for TA recording (Figure 1-A) [17]. The electrodes made of copper plate (5 mm square) were placed symmetrically on the dorsum of the tongue parallel to the midline for TP recording. They were 2 cm apart from each other at the posterior part of tongue on a line connecting the mandibular second molars. Since the adhesive could not function at this location, the electrodes were suspended by two orthodontic wires (0.6 mm in diameter and 35 to 45 mm in length) from an oral appliance (soft-elastic

rubber, 1 mm in thickness, BIOPLAST®, SCHEU-DENTAL, Germany), and were brought into contact with the tongue surface (Figure 1-B). The contact pressure of electrodes was adjusted for each subject by wire bending so that the contact pressure on the TP dorsum was adequate (approximately 10 g). Surface electrodes (NM-31, Nihon Kohden, Japan) were placed on the skin bilaterally over the digastric muscles for SHy muscle recordings. EMG signals were amplified (×1,000) and bandpass filtered (30-200 Hz) by custom-made amplifiers. They were then sampled at 1 kHz using 10-bit analog-to-digital converters of a micro-CPU (H8/3694; Renesas Electronics Corporation, Japan).

The signals (EMGs and BPs) were then entered into a personal computer, monitored on a screen, and logged on to a mass storage device in comma-separated



Figure 1: EMG electrodes and BP sensor settings

A: Recording sites for TA and TP. Assumed position of electrode for TP is indicated by "X."

B: The oral appliance for holding electrodes of TP and BP-o. BP sensor placed on center of ah-line. The EMG electrode for TP was suspended by two orthodontic wires from an oral appliance and was brought into contact with the tongue surface.

C: A schematic diagram of positions of the EMG electrode and BP sensor. The BP sensor for the oropharynx (BP-p) was inserted through the nasal cavity, and the setting position was confirmed by direct visualization via the oral side. Abbreviations: EMG, electromyography; BP barometric pressure; TA, anterior part of the tongue; TP, posterior part of the tongue, BP-o, BP in the oral cavity; BP-p BP in the pharynx; SHy, suprahyoid

values format. Stored data samples were later analyzed using analysis software (Spike 2; Cambridge Electronics Design Ltd. UK). Details of the BP sensor controller and EMG recorder have been described elsewhere [10,11,17,18].

3. Recording protocol

Since EMG electrodes for TA and TP were unique, the consideration of the EMG recording was tested first as follows: after setting the necessary EMG electrodes and the BP sensor, the study participants were asked to perform basic efforts for 5 sec each. These efforts included a tongue-pushing task (tongue press against the anterior hard palate near the maxillary central incisors) and a tongue-base lifting task (tongue-base lift like /ka/ production).

Two swallow events, as the main experiments, DS and 5 ml of water swallow (WS), were conducted separately without a random order. The study participants were asked to open their lips for 5 sec to record a base value for BP (i.e. atmospheric pressure) and then to close their lips for 5 sec, for DS. They were then instructed to swallow saliva and to hold that state for 5 sec as it was. Finally, the lips were opened for 5 sec. The subjects were asked to open their lips for 5 sec, for WS. Further, 5 ml of water was placed by a syringe on the dorsum of the tongue and the study participants were asked to hold that state for 5 sec. They were then instructed to swallow water asked to hold that state for 5 sec. They were then instructed to swallow water asked to hold that state for 5 sec. They were then instructed to swallow water and to hold that state for 5 sec. They were then instructed to swallow water and to hold that state for 5 sec. Finally, the lips were opened for 5 sec. These tests

were repeated three times for each subject

Due to the limitation of the recording system (four channels were the maximum for simultaneous recording) the experiment was separated into two sessions: i.e. first BP changes were recorded in the oral cavity (i.e. BP-o) and oropharynx (i.e. BP-p) during DS and WS, and then BP-o and the EMG activities of TA, TP, and SHy were recorded simultaneously. Measured parameters for BP-o and BP-p included times of onset, peak, and cessation and the peak values of BP-o and BP-p. BP-o value at the time of BP-p peak was further addressed. Times of onset, peak, and cessation were measured for EMGs. Only one researcher carried out these recording protocols and analyzed the measures. Therefore, intra-researcher reliability was not assessed.

4. Data analysis

The EMGs at TA and TP during the tongue-pushing and tongue-base lifting tasks were fully rectified and one second of accumulated data were compared between the tasks within the subject to check the reliability of TA and TP recordings.

The onset and cessation times of BP-o and BP-p were measured as follows; first, the mean and standard deviation (SD) were obtained from 5 sec of BPs during rest (i.e. atmospheric pressure). The onset was then defined as being when BPs were in excess of two SDs from base line; cessation was defined as being when BPs reduced across two SDs. Onset time, peak time, and cessation time for the TA, TP, SHy, and BP-o were measured after EMG bursts and were full-rectified and smoothed (time constant = 20 ms) using the same procedure as that for the BP measurements. The process was carried out with the Spike 2 (Cambridge Electronic Design Ltd, UK) software. Times of the onset, peak, cessation, and BP values were obtained using Microsoft Excel (Microsoft Inc, USA).

5. Statistical analysis

Differences in mean amplitude for TA and TP were analyzed using the Wilcoxon matched-pairs signed-rank test to evaluate the reliability of the TA and TP recordings. A p-value of < 0.05 was considered statistically significant.

Data in the swallowing tasks were first analyzed using conventional descriptive statistics, e.g., mean and SD. The differences between onset, peak, and cessation times among the TA, TP, SHy, and BP-o were analyzed using the repeated-measure one-way analysis of variance test followed by the Tukey's multiple-comparisons test. The statistical tests were performed using GraphPad Prism 6.0 h (GraphPad Software, USA).

<u>Results</u>

1. Consideration of the tongue EMG recordings

The raw data of one participant and the graphical comparisons of the two tasks for TA and TP are shown in Figure 2. Results show that TA recorded larger activities during the tongue-pushing task ($200 \pm 57 \ \mu V \cdot s$) than that during the tongue-base lifting task ($105 \pm 22 \ \mu V \cdot s$) (p = 0.002). Inversely, TP recorded larger activities during the tongue-base lifting task ($141 \pm 32 \ \mu V \cdot s$) than that during tongue-pushing task (70 $\pm 27 \ \mu V \cdot s$) (p = 0.002) (Figure 2).



Figure 2: *Tongue muscle activities during tongue pushing and tongue-base lifting tasks* An example of EMG recordings in TA (upper left) and TP (lower left) for each task and graphical comparisons of TA and TP between the two tasks (upper right and lower right). The EMGs were fully rectified and one second of accumulated data was compared between the tasks. Statistical analysis was conducted using the Wilcoxon matched-pairs signed-rank test (**p < 0.01).

Abbreviations: EMG, electromyography; TA, anterior of the tongue; TP, posterior of the tongue

2. The relationship between BP-o and BP-p

An example of the simultaneous recording of BP-o and BP-p during DS and WS is shown in Figure 3, in which arrows ("a through "e") mark notable inflection points. Measured timings and peak pressures are listed in Table 1. BP-p stayed at the atmospheric pressure level before and after the large positive pressure changes ("a" through "e"). However, BP-o before the large positive pressure change (before point "a") was often slightly lower or higher than the atmospheric pressure level especially during DS.



Figure 3: An example of recordings of the BP changes in the two cavities during DS and WS Changes in BP-o and BP-p are shown by gray and black lines, respectively. Characteristic change sites are indicated by arrows "a" through "e"; arrow "a" indicates the beginning time of increment of BP-o and BP-p, arrow "b" indicates the peak time of BP-o, arrow "c" indicates the peak time of BP-p, arrow "d" indicates the time of BP-o at the peak time of BP-p, and arrow "e" indicates the return time of BP-o and BP-p to the baseline. BP-o peaked immediately after onset and decreased to atmospheric pressure after a short plateau. BP-p gradually increased, reached a peak, and returned to atmospheric pressure immediately before the end of BP-o.

Abbreviations: BP barometric pressure; DS, dry swallow; WS, wet swallow; BP-o, BP in the oral cavity; BP-p BP in the pharynx

	DS		WS		Reference	
	BP-o	BP-p	BP-o	BP-p	point	
Onset (sec)	-0.21±0.10	-0.16±0.08	-0.14±0.09	-0.07±0.04	'a'	
Peak (sec)	Reference time	0.15±0.09	Reference time	0.34±0.11	'b' and 'c'	
Cessation (sec)	0.43±0.11	0.47±0.15	0.51±0.08	0.55±0.19	'e'	
Peak value (kPa)	117.6±3.5	116.2±4.4	115.6±4.4	113.6±4.1		
BP value at peak time of BP-p (kPa)	115.2±4.6		111.5±4.8		'd'	
Duration (sec)	0.64±0.11	0.63±0.14	0.65±0.12	0.62±0.13	'a' to 'e'	

Table 1: Timings of BP changes during DS and WS

The peak BP-o was used as the reference for onset, peak, and cessation times. Values in the table are expressed as mean \pm SD (n = 10). Reference points in table 1 are the same as points 'a' to 'e' in figure 2.

At the point "a," both BP-o and BP-p began to increase. Especially, BP-o

increased sharply towards the peak (point "b"), slowly fluctuated until point "d," and gradually returned to the atmospheric pressure level at "e." BP-p also increased after point "a." It increased towards the peak (point "c") via a plateau or slow changes and returned to the atmospheric pressure level at point "e." In the case of BP-p, a small negative pressure was obtained in about 63% (38 of 60) of all trials just before point "e."

After the peak of BP-o (point "b"), two curves crossed so that BP-p became higher than BP-o. The peak time of BP-o was used as the reference for onset, peak, and cessation times in the Table 1 because the BP-o waveform showed a clear peak and could easily be used as a reference. Peak time of BP-o and onset of BP-p were small in its variance and were close in the absolute value to each other; especially, that of BP-p during WS was close (the difference was only 0.07 sec) to the reference.

3. Timings of activity of swallow related organs during DS and WS

An example of recorded raw data and a feature of activity timings is illustrated in Figure 4, and the mean and SD of onset, peak, and cessation are shown in Table 2

Mean onset times were obtained in the order of TA, SHy, TP, and BP-o. The onset times of TP and SHy were close in both DS and WS, and the onset time of TA preceded that of TP by 0.06 sec and 0.05 sec in DS and WS, respectively. The onset time of TA preceded that of SHy by 0.03 sec and 0.05 sec for DS and WS, respectively. There were significant differences between onset time of TA and onset time of TP for WS (p = 0.018).

Mean peak times were obtained in the order of TA, TP, and SHy/BP-o in both DS and WS. The peak of TA activity preceded that of BP-o in both DS (p = 0.047) and WS (p = 0.045). The peak of TP activity also preceded that of BP-o in DS (p = 0.038) and that of SHy in WS (p = 0.043). In the case of WS, the peak of TA activity preceded that of SHy (p = 0.011).

Mean cessation times were obtained in the order of TA, TP, SHy, and BP-o. Statistically significant differences were seen between TA and SHy (p = 0.022) for DS and between TA and BP-o (p = 0.047) for DS and WS.





A: An example of recordings of EMG activities and BP-o in WS.

B: The temporal pattern of EMG activities and BP-o change. Peak BP-o was defined as the reference time. Means ± SD (n = 10) of the onset, peak, and cessation times for DS and WS are presented. Abbreviations: EMG, electromyography; BP barometric pressure; TA, anterior part of the tongue; TP, posterior part of the tongue, BP-o, BP in the oral cavity; BP-p BP in the pharynx; Shy, suprahyoid; DS, dry swallow; WS, water swallow

		ТА	TP	SHy	BP-o
DS	Onset	$\textbf{-0.39}\pm0.13$	$\textbf{-0.33}\pm0.08$	$\textbf{-0.36} \pm \textbf{0.12}$	-0.20 ± 0.11
	Peak	-0.16 ± 0.12	$\textbf{-0.14}\pm\textbf{0.10}$	$\textbf{-0.04} \pm \textbf{0.12}$	Reference time
	Cessation	$\textbf{0.22}\pm\textbf{0.11}$	$\textbf{0.36}\pm\textbf{0.11}$	$\textbf{0.36} \pm \textbf{0.07}$	$\textbf{0.43}\pm\textbf{0.10}$
WS	Onset	$\textbf{-0.45} \pm \textbf{0.09}$	$\textbf{-0.40}\pm\textbf{0.09}$	$\textbf{-0.40} \pm \textbf{0.10}$	-0.19±0.08
	Peak	$\textbf{-0.14} \pm \textbf{0.10}$	-0.10 ± 0.11	$\textbf{0.02}\pm\textbf{0.10}$	Reference time
	Cessation	$\textbf{0.22}\pm\textbf{0.15}$	$\textbf{0.28}\pm\textbf{0.11}$	$\textbf{0.34}\pm\textbf{0.11}$	$\textbf{0.45}\pm\textbf{0.10}$

Table 2: *Timings of EMGs (TA, TP, and SHy) and BP-o change during DS and WS* The peak of BP-o was used as the reference for onset, peak, and cessation times. Values in the table are expressed as mean \pm SD (n = 10).

Abbreviations: EMG, electromyography; BP barometric pressure; TA, anterior part of the tongue; TP, posterior part of the tongue, BP-o, BP in the oral cavity; BP-p BP in the pharynx; Shy, suprahyoid; DS, dry swallow; WS, water swallow, SD, standrad deviation

Discussion

1. Methodological considerations of TA and TP recordings

Since a wire held the electrode for TP, motion artifact and inconsistence contact could be anticipated. We tested the pressure of electrode on the tongue to reduce the motion artifact and inconsistence contact. We decided the best contact pressure as 10 g. According to the report of Ono et al. [6] the pressure on the palate at the tongue base was 19 kPa \pm 5 that is equivalent to 95 g for the small electrode and could be enough to fix them on the tongue surface. Further, to reduce tongue movement during swallowing, the volume of water was set to 5 ml, which the subjects could swallow at one time. Thereafter, as shown in Figure 4A, most of the recordings were free from artifacts.

EMGs at TA and TP were recorded using surface electrodes. TA showed two times larger activity than the tongue lifting base task during the tongue-pushing task, and conversely, TP showed two times larger activity than the tongue-pushing task during the tongue-base lifting task (see Figure 2). These results indicate that the electrodes at TA and TP could record activities of the different muscles at the anterior and posterior portions. Kumada et al. [19] used MRI to measure the length of intrinsic tongue fibers and the GG muscle when producing the sounds /t/ and /ka/ (equivalent to the tongue-pushing and tongue-base lifting tasks, respectively). They found that the superior longitudinalis and the anterior part of the GG muscles synergistically move the tongue-body antero-inferiorly, and they antagonistically move the tongue tip antero-superiorly/postero-inferiorly. Furthermore, Napadow et al. [20] had used MRI to study a lingual muscular deformation during swallowing. They reported a verticalis contraction, which was related to the expansion of the tongue tip towards the incisors maxillary. The contraction was seen in the early stage, and the grooved depression appeared to have been created by a contraction of the anterior GG in combination with the hyoglossus, verticalis, and transversus muscles. Although there is no direct evidence for this, the electrodes at TA and TP may record the intrinsic tongue muscle activities.

2. Comparison of BPs in the two cavities

From the series of studies conducted, we have shown the usefulness of BP measurement in the evaluation of oral functions [10,11,17,18]. Hasegawa et al. [11] placed a BP sensor in the oropharynx and obtained the BP changes that occurred during swallowing. Although the BP sensor is small and the placement technique is similar to that for a video endoscopy, the process of its placement in the oropharynx through the nasal cavity may not be comfortable for the patients. If BP-o measurements (i.e. oral side approach of BP measurement) give information, which is equivalent to that of BP-p measurements, then this could constitute a preferable tool

for the assessment of swallow for both the examiner and patient. In this study, we measured BP-o and BP-p simultaneously during DS and WS, and found a reference time at the BP-o peak, which was close to the BP-p onset. The timings of the EMG activities and the BP changes will be discussed sequentially point by point ("a" through "e" indicated in Figure 3) based on the reference time.

2-1 Oral stage before swallowing (before point "a")

In this study, TA was the first activity observed during swallowing by both EMG activities and BP changes. The oral stage of swallowing starts with the accumulation of the food bolus by the tongue and ceases with the onset of nasopharyngeal closure. At the beginning, the tongue scoops the bolus to a supralingual position. With this motion, the tongue tip reaches the posterior aspect of the maxillary incisors [3].

BP changes cannot be observed unless the space at the sensors is isolated from the atmospheric environment. Some fluctuations seen during WS around "a" could be understood as occurring due to the fact that the oral cavity was isolated from the outside at jaw rest position [9], since the lips had been closed and the tongue-palate gap had been sealed to hold water in the oral cavity. Studies by VF [3,4] and tongue contact force measurement [6] may support the following event sequence: as the tongue begins to protrude food is pressed against the palate using sequential contact by tongue at the tip, tongue movements squeeze the space, and then nasopharyngeal closure occurs [2,4]. During DS, small fluctuations due to the collection of saliva by the tongue were observed. It could be suspected that just before "a" the tongue and soft palate made tight contact and the food bolus protrusion began i.e. the oral stage of swallow started. Thus, the duration of the oral stage of swallowing can be defined as the time between the onset of TA and the BP-o peak. In fact, the durations of 0.39 \pm 0.13 sec for DS, 0.45 \pm 0.09 sec for WS obtained in this study are close to those (0.38 \pm 0.05 sec) reported by Okada et al. [4] using VF.

As to the timings of the activities of TA and TP, Dodds et al. [3] have shown that the anterior two-thirds of the tongue elevates as a globular mass to make sequential peristaltic contact with the hard palate after the tongue tip scooping movement. Thus, the TA may begin the activity shortly before the TP onset for making sequential peristaltic contact. Since the above movement is followed by a nasopharyngeal closure, peaks of TA and TP may precede the nasopharyngeal closure. Our results relating to TA and TP support this, onset times were in the order of TA, SHy, TP, and BP-o; peak times were in the order of TA, TP, and SHy/BP-o (see Figure 4-B). The intrinsic tongue muscle activity (TA and TP) may reach its peak before the onset of nasopharyngeal closure.

Since the EMG activity in submental muscles most often initiates the swallow [14], many studies have recorded SHy activity as a reference point [14,15,21]. Palmer

et al. [22] reported that the primary contributors to submental surface recordings were the mylohyoid, anterior belly of the digastric, and the geniohyoid muscles (i.e. the SHy muscle), and that contributions from the GG and the platysma muscles were minimal. In addition, Tsukada et al. [15] recorded GG and SHy activity during swallowing, and reported that the onset and cessation times of the GG muscle preceded those of the SHy. These results may be interpreted as; the TA is active before the SHy becomes active. In this study, the onset time of TA preceded that of SHy in DS and WS. However, it should be noted that there were no significant differences. It should be stressed that the peak time of SHy is around the BP-o peak, i.e. the time at onset of the nasopharyngeal closure. The finding is supported by the study of Hasegawa et al. [11].

The muscle at the pharyngoesophageal junction is tonically active at rest and relaxes during swallowing, belching, and vomiting. Its main function is to control anterograde and retrograde flow of contents between the pharynx and esophagus [23]. Celik et al. [24] studied this phenomenon electromyographically. According to their study, the cricopharyngeal muscle is suppressed for approximately 0.5 sec after the peak time of the SHy during swallowing. This suggests nasopharyngeal closure and relaxation of the upper esophageal sphincter may occur in the same timing (i.e. onset and duration).

2-2 Start of swallowing to peak of pharyngeal contraction (point "a" through "c" or "d")

At the point "a," BP-o began to increase, and BP-p began to increase shortly after BP-o. At this stage, the oral cavity was isolated by the lip seal and by tongue-palate contact. Additionally, the oropharynx was isolated by nasopharyngeal closure. BP-o peaked at "b," which indicated the oral cavity was compressed maximally. This occurs at the time when the tongue and palate mostly close each other and the space in the oral cavity may begin to squeeze the food. At this moment, a part of the food bolus can be pushed into the oropharynx, i.e. the seal between oral cavity and pharynx partly breaks [25]. If this is the case, the BPs in the two cavities should be equivalent. However, the peak value of BP-p is less than the BP-o at the peak time of BP-p, forming a small peak or plateau. We believe that this difference may be explained by the fact that the food bolus itself works as a septum for the two spaces. Hasegawa et al. [11] reported the same results and concluded that the small peak could be generated, at least in part, by the driving force of the tongue against the palate at the maximum level. In addition, Jones et al. [12] reported a similar pattern in the pharynx, using an endoscopically shaped high-resolution manometry that could measure pharyngeal and esophageal BPs during swallowing. Therefore, it can be concluded that the oral and pharyngeal phases of swallowing can be distinguished by the peak time of BP-o, when the nasopharyngeal closure begins.

2-3 End of swallowing (point "e")

After the peak, BP-p value decreased rapidly towards "e." At this moment, both BP-o and BP-p returned to atmospheric level simultaneously. This could be understood by the fact that although the lips are closed at the time of "e," nasopharyngeal closure ceases. Therefore, the upper esophageal sphincter may open and the food bolus may come into the esophagus [4]. Thus, BP-p decreases. In some cases, during WS there was negative pressure just before point "e." Hasegawa et al. [11] has described this negative pressure. They concluded that the negative pressure induced after positive BP could reflect the relaxation of the oral and pharyngeal muscles during swallowing. It could be concluded that point "e" is the time at which nasopharyngeal closure ceases and thus, the pharyngeal phase of swallowing ends.

3. Nasopharyngeal closure

Since the pharynx is in contact with the outside of the body via the nasal cavity and/or oral cavity, the BP-p value should be equivalent to the atmospheric pressure unless the cavity is isolated. Thus, the time at the onset of the BP-p increase may be the onset of nasopharyngeal closure. Conversely, the time at BP-p cessation may be the end point of the closure. The BP-p onset time was measured as - 0.07 ± 0.04 ms, which is close to the reference time (i.e. peak time of BP-o). The cessation times for BP-o and BP-p were close to each other $(0.51 \pm 0.08 \text{ ms} \text{ and } 0.55 \pm 0.19 \text{ ms},$

respectively). Thus, the duration of the closure is measured as approximately 0.6 ms in this study. Okada et al. [4] reported similar results using VF; however, they could not show precise timings for nasopharyngeal closure. Therefore, it is concluded that the onset and cessation times of nasopharyngeal closure can be measured with higher resolution in the BP-o.

Mean cessation times were in the order of TA, TP, SHy, and BP-o, both during DS and WS. TA activity ceased earlier than the others with the exception of TP in WS. BP-o ceased at the same time as BP-p, i.e. when the pharyngeal stage of swallow might end. The end of the pharyngeal stage corresponds to that of the infrahyoid muscle activity [13]. We now know that one barometric sensor in the oral cavity and one muscle (suprahyoid) are enough to study the durations of oral and pharyngeal phases of swallowing. Thereafter, we will expand the number of subjects and will test foods with different consistencies.

<u>Conclusion</u>

In this study, a clear reference point for the discussion of swallow events was obtained using BP-o peak time. BP measurements revealed swallow related events in the oral cavity and oropharynx with high time resolution. Our findings show that (i) intrinsic tongue muscle activity might reach the peak before the onset of nasopharyngeal closure (ii) the BP-o peak time is equivalent to the time of the onset of nasopharyngeal closure, (iii) the duration of the oral stage of a swallow may be obtained from the time between the onset of TA and the BP-o peak time as 0.4 sec, (iv) the BP-o and/or BP-p cessation time is the time at the termination of nasopharyngeal closure, and (v) the duration of the pharyngeal stage of the swallow may be obtained from the time between BP-o peak time and BP-o and/or BP-p cessation time as 0.6 sec.

BP-o measurement can separate the oral and pharyngeal stages, so this noninvasive technique can be easily used for research on swallowing.

<u>Disclosure</u>

The authors declare no conflicts of interest associated with this manuscript.

28

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29

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