The Laryngoscope

Upper Esophageal Sphincter Response to Laryngeal Adductor Reflex Elicitation in Humans

Frederik J. Meisoll, DMD[†]; Michael Jungheim, MD[†]; Jacob F. Fast, MSc; Simone Miller, PhD; Martin Ptok, MD

Objective: The laryngeal adductor reflex (LAR) is an important mechanism to secure the airways from potential foreign body aspiration. An involvement of the upper esophageal sphincter (UES) in terms of a *laryngo-UES contractile reflex* has been identified after laryngeal mucosa stimulation. However, the LAR–UES relationship has not yet been fully explained. This study aimed to determine the magnitude, latency, and occurrence rate of the UES pressure response when the LAR is triggered in order to elucidate the functional relationship between the larynx and the UES.

Methods: This prospective study included seven healthy volunteers (5 female, 2 male, age 22–34 years). Laryngeal penetration was simulated by eliciting the LAR 20 times in each individual by applying water-based microdroplets onto the laryngeal mucosa. UES pressures were measured simultaneously using high-resolution manometry.

Results: Two distinct pressure phases (P1, P2) associated with the LAR were identified. P1 corresponded with a short-term UES pressure decrease in two subjects and a pressure increase in five subjects occurring 200 to 500 ms after the stimulus. In P2, all subjects experienced an increase in UES pressure with a latency time of approximately 800 to 1700 ms and an average of 40 to 90 mmHg above the UES resting tone.

Conclusion: Foreign bodies penetrating the laryngeal inlet lead to a reflex contraction of the UES. Phase P1 could be a result of vocal fold activity caused by the LAR, leading to pressure changes in the UES. The constriction during P2 could strengthen the barrier function of the UES in preparation to a subsequent cough that may be triggered to clear the airways.

Key Words: Upper esophageal sphincter, laryngeal adductor reflex, high-resolution manometry, laryngo-UES contractile reflex, airway-protective mechanism.

Level of Evidence: 4

***Laryngoscope*, 00:1–7, 2020**

INTRODUCTION

Aspiration pneumonia is a potentially life-threatening condition caused by penetration of foreign material into the laryngeal inlet and consecutive aspiration into the deeper airways. This may be caused by a dysfunctional swallowing process,[1] regurgitation of esophageal or gastric contents,[2] and aspiration of foreign material, which might be accidentally drawn into the respiratory tract while breathing. There are several laryngeal protective mechanisms to prevent aspiration,[3] including the laryngeal adductor reflex (LAR).[4]

The LAR is widely understood as a contractile reflex induced by the lateral cricoarytenoid and the transverse and oblique arytenoid muscles, which close the glottis by adduction of the vocal folds.[5] It is triggered by mechanical[4] or electrical[6] stimulation of the laryngeal mucosa. To test the reflex clinically, air pulse stimuli can be applied. This is known as the *fiberoptic endoscopic evaluation of swallowing with sensory testing* (FEESST) method.[7] Alternatively, small water-based droplets, as in *microdroplet impulse testing of the LAR* (MIT-LAR),[8,9] or electrical stimulation of the internal branch of the superior laryngeal nerve[10] have been proposed in previous studies for controlled LAR elicitation. Two separate adductive components of the LAR have been identified. An early LAR1 was identified to occur within 8 to 18 ms following a trigger event in anesthetized cats and dogs.[5] Recently, the bilateral nature of the human LAR1 was observed.[11] The human LAR2 response occurs within approximately 65 to 70 ms following electrical stimulation of the superior laryngeal nerve[10] and 106 ± 43 ms (mean ± standard deviation [SD]) after stimulation of the laryngeal mucosa by microdroplet impact.[8] Due to the short interval of vocal fold adduction, the LAR can only temporarily prevent the bolus material from being aspirated. To remove

From the Department of Phoniatrics and Pediatric Audiology (F.J.M., M.J., J.F.F., S.M., M.P.), Hannover Medical School, Hannover, Germany; Institute of Mechatronic Systems (J.F.F.), Leibniz Universität Hannover, Hannover, Germany.

Editor's Note: This Manuscript was accepted for publication on September 18, 2020

[†]These authors contributed equally to this work.

Presented at the 36th annual conference of the "Deutsche Gesellschaft für Phoniatrie und Pädaudiologie" (DGPP, German Society of Phoniatrics and Pediatric Audiology), Göttingen, Germany, September 22, 2019.

Parts of this project were funded by Deutsche Forschungsgemeinschaft, grants PT 2/5-1 and KA 2975/6-1. J.F.F. and M.P. filed patents related to MIT-LAR that are currently under review. These two authors therefore declare a potential conflict of interest. The authors have no other funding, financial relationships, or conflicts of interest to disclose.

Send correspondence to Michael Jungheim, Department of Phoniatrics and Pediatric Audiology, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany. E-mail: jungheim.michael@mh-hannover.de

DOI: 10.1002/lary.29166

material from the supraglottic space, additional mechanisms such as coughing are necessary.

The protection of the larynx also involves the upper esophageal sphincter (UES), which is anatomically connected to the larynx and shares motor innervation by the recurrent laryngeal nerve. Therefore, overlaps in the function of both organ systems can be expected. The sphincter is composed of the thyropharyngeus and cricopharyngeus muscle as part of the inferior pharyngeal constrictor and superior parts of the esophagus.[12,13] These muscles and additional elastic fibers maintain a resting pressure in order to keep the esophagus closed at its entrance.[14,15] Besides preventing regurgitation of swallowed material back into the pharynx, the UES inhibits accidental inhalation of air into the esophagus during breathing.[16] It plays a major role in separating the swallowing path from the airways.

Regarding UES functions, it is known that resting pressures change in response to pharyngeal[17] or esophageal[18,19] stimuli. Furthermore, vocal fold movement in phonation induces the *phonation-induced UES contractile reflex*.[20] In addition, a *laryngo-UES contractile reflex* has been identified,[21] which can be considered part of the airway protection. This reflex was triggered when air pulses were applied to the arytenoids and interarytenoid area.[21] Elicitation reliability is said to depend on stimulus duration and intensity[22] and was found more difficult to be triggered in the elderly.[23]

However, the studies conducted thus far were not able to fully clarify the relationship between a laryngeal stimulation and a UES response. The specific response by the UES when the LAR is triggered remains unclear. There is also a lack of information about the exact pressure changes and temporal parameters, such as the reflex latencies. Therefore, this study aimed to 1) determine the magnitude of UES pressure changes when triggering the LAR, 2) evaluate the latency, and 3) evaluate the occurrence rate of UES responses to elucidate the functional relationship between the larynx and the UES.

METHODS

Study Type

This was a prospective, single-center, experimental study including healthy volunteers.

Volunteers

Seven adults (5 female and 2 male, age range 22–34 years) were included in this study. Inclusion criterion was a good state of health. Exclusion criteria were a medical history of dysphonia, pregnancy, diseases of the larynx or the esophagus, neck or esophageal surgery, muscle disease, and age above 60 years to avoid cases of subclinical dysphagia.

High-Resolution Manometry

High-resolution manometry (HRM) was performed similarly to previous investigations[24–26] to evaluate pressure parameters of the UES. Data were collected using a solid-state HRM hardware system (Solar GI HRM, Medical Measurement Systems [MMS], Enschede, The Netherlands) with a manometric catheter (Unisensor, Attikon, Switzerland) specifically designed to measure pharyngeal and UES pressures. The catheter had an outer diameter of 2 mm and a total of 20 unidirectional pressure sensors, of which 19 were spaced at intervals of 7.5 mm and the one remaining sensor was placed at the end of the catheter. The catheter was calibrated and sterilized according to the manufacturer's specifications before each measurement. All pressures were recorded in reference to atmospheric pressure and body temperature. Data were acquired at a frequency of 50 Hz for each sensor. The collected data were analyzed using MMS software v 8.20e (MMS Database) after adjustment to the abovementioned catheter.

Microdroplet Impulse Testing of the LAR

A microdroplet application device specifically designed for clinical studies was used to ensure a controlled elicitation of the laryngeal adductor reflex. The safety of this system has been demonstrated previously.[9] The droplet applicator module consisted of a solenoid valve (The Lee Company, Westbrook, CT) operated electronically and connected to a metal tube bent at a 90° angle. A water reservoir, pressurized at 1.2 bar with respect to the atmospheric pressure, was connected upstream of the valve. The valve was opened for 20 ms, and a droplet was released at the distal end of the nozzle. In this way a constant volume of the ejected fluid was guaranteed. Food colorant E 150c (Appel Feinkost GmbH & Co. KG, Cuxhaven, Germany) was added at a concentration of 1:20 (V/V) to enhance visibility of the droplet in the laryngoscopic images.

The LAR was triggered by the droplet applicator module mounted to a laryngoscopic high-speed glottography (HSG) system (HRES Endocam 5562, Richard Wolf GmbH, Knittlingen, Germany) while a high speed laryngoscopic sequence was recorded (Fig. 1). To reduce the dimensions of the applicator module compared to the original MIT-LAR prototype system, a custom mount was designed and manufactured using stereolithography to place the valve in extraoral proximal position on the laryngoscope shaft. The HSG system provided a framerate of 4000 fps and allowed saving previously recorded sequences on an external hard drive. A video sequence, containing the flight of the microdroplet, impact on the laryngeal mucosa, and resulting adductive motion of the vocal folds, was saved by pressing a button on the handle of the laryngoscope directly after LAR activity was visually detected.

Synchronization of HRM and MIT-LAR

To determine the exact time of microdroplet impact and initial LAR motion in the corresponding manometry sequence, an electronic interface module for the HRM and the MIT-LAR system was used. The device provided a voltage supply to an auxiliary channel in the manometry system that was grounded when poles short-circuited. This occurred when the glottography recording stopped, producing an output signal that was registered by the interface module. This signal was transferred to the manometry software without time delay so that events such as droplet impact and initial vocal fold adduction could be inserted into the manometric timeline.

Procedures

While the volunteers sat in an upright position with the head in neutral position, the manometric catheter was placed transnasally into the upper esophagus and fixed in place at the tip of the nose. No lubricating gel containing a local anesthetic

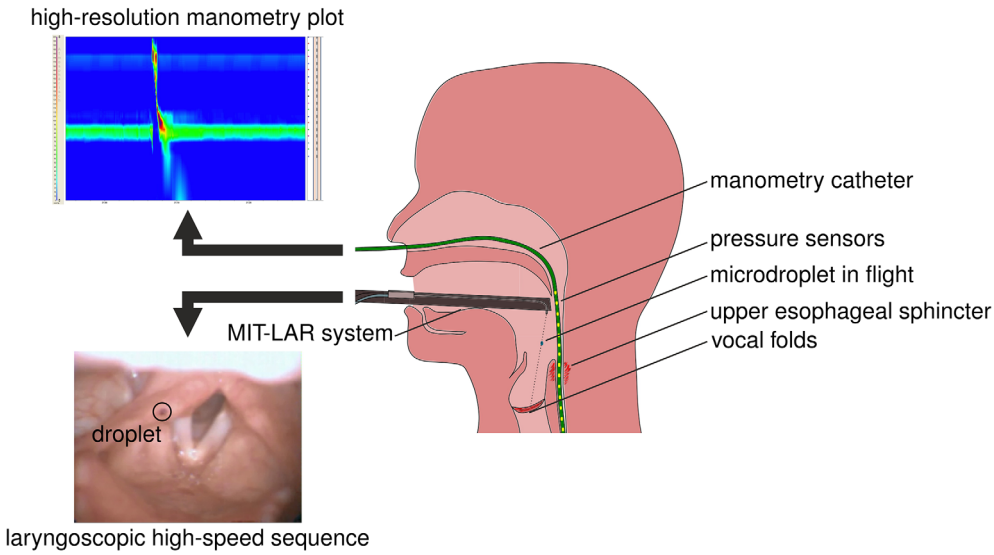

Fig. 1. Testing results in two data sets: high resolution manometry data and high-speed image data. MIT-LAR = microdroplet impulse testing of the laryngeal adductor reflex. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

agent was used in order to prevent any alteration of mucosal sensitivity. The small-diameter catheter was positioned properly to cover the high-pressure area of the UES and was taped to the participant's nose to avoid displacement during the experimental session. Each participant rested for at least 10 minutes in order to become accustomed to the catheter. Testing began as soon as the UES showed a constant resting pressure without any disruptions caused, for example, by swallowing. The endoscope with the microdroplet applicator module was inserted transorally into the pharynx. As soon as the glottal plane was visible, the valve was opened once to eject the defined fluid volume to stimulate the laryngeal mucosa. Regions that are known for providing high-reflex activity, such as arytenoids, interarytenoid area, and vocal folds, were specifically stimulated.[27] In case of no detectable LAR, another fluid volume was released aiming at a slightly different region within the larynx because a successfully triggered LAR was required to investigate the research question of this study. In case of LAR activation, video recording was stopped as soon as the adductive motion of the vocal folds was demonstrated. Video material from the glottal area and manometry recordings were saved after each successfully elicited LAR. This process was repeated until a data set including 20 LAR events was achieved for each subject. If a retching reflex or a swallow was triggered, the corresponding data was discarded, and the test was repeated.

Data Acquisition

HRM pressure sensors corresponding to the high-pressure zone of the UES were defined for each participant. UES responses following the successful release of the LAR were identified individually for each test within the high-resolution manometry spatiotemporal plot.[28]

In case of an identifiable UES response in terms of a pressure change compared to the resting pressure, the following time parameters were noted:

Time interval TI between microdroplet impact and beginning of pressure deviation from UES resting pressure

Time interval TO between LAR onset and beginning of pressure deviation from UES resting pressure

Time span TS of pressure deviation from UES resting pressure

The following manometric parameters were calculated for each UES response and compared to UES resting pressure (RP), which was averaged over a time period of 1 second before stimulation:

Mean pressure MP over the time span of each UES response

Peak pressure PP within the time span of each UES response

For each of the temporal and manometric parameters above, intraindividual averages and SDs were calculated using SPSS 25 (IBM Corp., Armonk, NY).

Compliance with Ethical Standards

Ethical approval: The study was performed in accordance with the 1964 Declaration of Helsinki and its later amendments, Good Clinical Practices, and applicable regulatory requirements. The study was approved by the ethics committee of Hannover Medical School (3145/2016).

Informed consent: All participants signed an informed consent form before undergoing any study-related procedures and were not financially remunerated.

RESULTS

Tests were completed in all seven participants without any complications. There were no complaints of any relevant discomforts caused by the MIT-LAR or HRM procedure, and none of the test series had to be discontinued.

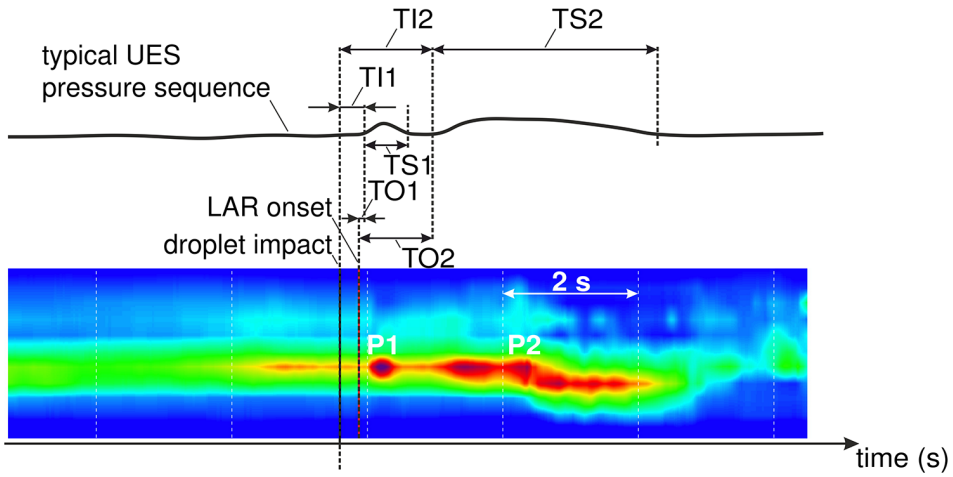

Fig. 2. Representative temporal evolution of upper esophageal sphincter pressure after laryngeal adductor reflex activation and corresponding high-resolution manometry spatiotemporal plot (TI1/TI2: time interval between droplet impact and beginning of P1/P2; TO1/TO2: time interval between LAR onset and beginning of P1/P2; TS1/TS2: duration of P1/P2). LAR = laryngeal adductor reflex; P = pressure phase; UES = upper esophageal sphincter. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

Two distinguishable UES responses P1 and P2 (Fig. 2) were identified in each participant when manometry data was evaluated:

P1: An early and short-duration UES response was detected following elicitation of the LAR with an occurrence rate of 50% to 85% (Table I). The latency period TI1 following the impact of the microdroplet varied between 282 ± 75 ms and 467 ± 96 ms. This change in UES pressure was found to be either a pressure rise of approximately 33 to 47 mmHg (5 of 7 participants) or a pressure drop of approximately 10 to 15 mmHg (2 of 7 participants) with a time span TS1 of 230 ± 100 ms to 850 ± 610 ms (Table II).

P2: A longer-lasting late contractile UES response was detected in all seven individuals with an occurrence rate of 40% to 95% and a latency TI2 of 812 ± 343 ms to 1661 ± 230 ms following microdroplet impact. P2 was measured as a pressure rise of approximately 40 to 90 mmHg in each individual participant (Table III) and had a duration TS2 of 1110 ± 240 ms to 2230 ± 590 ms.

P1 and P2 occurred with a higher latency than the LAR in all participants and either occurred both in one trial (P2 following P1) or as a single pressure deviation.

TABLE I.
Occurrence Rate of Pressure Phases P1 and P2 Resulting from n = 20 Trials per Individual

Participant No.	P1 Occurrence rate	P2 Occurrence Rate
1	50%	75%
2	80%	95%
3	55%	70%
4	75%	55%
5	85%	60%
6	65%	40%
7	70%	65%

P = pressure phase.

An early LAR1 component could not be detected visually in any of the experiments. For LAR2 onset latency, an interindividual mean value of 194.4 ± 89.4 ms to 324.5 ± 121.6 ms was determined (Table IV).

DISCUSSION

In this study, we investigated the behavior of the UES when the LAR was triggered by microdroplet impact on the laryngeal mucosa. The main finding was a UES response composed of two separately occurring pressure deviations (P1 and P2) from the UES resting pressure.

Compared to UES resting pressure, P1 appeared as a pressure drop in two subjects and an increase in five subjects. Multiple factors may influence UES pressure during the early P1 response. When the LAR is triggered, a vocal fold closure occurs. In a previous study of 13 healthy human subjects, the average duration of the bilateral vocal fold adduction phase and the glottic closure phase in LAR were found to be approximately 110 ms and 200 ms, respectively.[29] In the present study, the onset of pressure phase P1 occurred approximately 116 ms after the onset of vocal fold adduction on average. This indicates that the incidence of full glottic closure and P1 onset are closely related in time. Furthermore, P1 was found to be terminated on average 518 ms after LAR onset. Shock et al. reported a mean overall LAR duration of approximately 550 ms.[29] This also suggests an immediate influence of LAR activity on P1. A reason for the corresponding pressure increase seen in five subjects might lie in the parallel innervation of the larynx and the UES, which both are partially supplied by the recurrent laryngeal nerve.[30,31] Therefore, during vocal fold adduction, a simultaneous contraction of the muscles in the UES with a corresponding increase in pressure may have occurred. The short-term reduction of pressures in the UES observed in two participants may be related to the rotation of the arytenoid cartilage. During the LAR, the arytenoids move medially and anteriorly, leading to an

TABLE II.
Temporal Analysis of P1 and P2 (Mean ± SD)

	P1			P2		
Part. No.	TI1(ms)	TO1 (ms)	TS1 (ms)	TI2 (ms)	TO2 (ms)	TS2 (ms)
1	367 ± 108	170 ± 89	380 ± 150	1661 ± 230	1542 ± 287	1490 ± 490
2	354 ± 117	109 ± 62	240 ± 60	1261 ± 254	1022 ± 229	1280 ± 200
3	284 ± 96	95 ± 51	240 ± 40	1487 ± 258	1296 ± 250	1110 ± 240
4	282 ± 75	76 ± 45	230 ± 100	812 ± 343	607 ± 316	2230 ± 590
5	359 ± 141	126 ± 70	350 ± 120	1234 ± 398	983 ± 337	1860 ± 540
6	312 ± 95	111 ± 69	850 ± 610	858 ± 188	625 ± 162	1630 ± 620
7	467 ± 96	126 ± 77	520 ± 380	1651 ± 257	1349 ± 210	1460 ± 370

TI1/TI2: time interval between droplet impact and beginning of P1/P2.
TO1/TO2: time interval between LAR onset and beginning of P1/P2.
TS1/TS2: Duration of P1/P2.
P = pressure phase.

expansion of the space behind the larynx. If the anterior movement component is particularly pronounced, for example, due to contraction of the thyroarytenoid muscle and vocalis muscle, a reduction in pressure could result. This can vary inter-individually because the dynamic response pattern of the larynx may differ when the LAR is triggered. In principle, a laryngeal elevation, as observed during swallowing, could also lead to an expansion of the hypopharyngeal space and thus to a reduction of pressure in the upper esophageal sphincter.[32] However, this can be ruled out because no laryngeal elevation was observed endoscopically, and examinations in which a swallowing process was triggered were discarded. Additional investigations on a larger group of participants will be necessary to further assess this phenomenon.

P2 corresponded to a pressure increase in all subjects, which lasted longer than P1. It was seen and could be reproduced intra-individually in 40% to 95% of the experiments. Because P2 occurred after the beginning of vocal fold movement or after it was already completed, and because P2 also lasted longer than the vocal fold activity, a direct influence of vocal fold activity on P2 is not plausible. P2 is more likely to be caused by a reflexive contraction of the UES muscles. The presence of a *laryngo-UES-contractile reflex*[20] can be confirmed with the investigations of this study.

Kawamura et al. noted a similar behavior of the UES as a consequence of laryngeal stimulation.[23] In their study, a UES pressure increase, which probably corresponds to P2, was measured when air pulses were applied to the laryngeal mucosa. Trifan et al. also reported a UES pressure increase after pharyngeal stimulation with a small volume of water while an inhibitory action on the lower esophageal sphincter was observed.[33] However, a UES reaction that includes two temporally distinguishable responses as observed in the present study has not been reported so far.

Regarding the reflex arc, it is not yet clear how laryngeal afferents are processed in the central nervous system. Because laryngeal and UES muscles are both partly innervated by the vagus nerve, it may be possible that central processing occurs within the same regions of the nervous system, resulting in a common efferent signal for both organs.[34] A joint reaction of vocal folds and UES

TABLE III.
Manometric Analysis of P1 and P2 (Mean ± SD)

	P1			P2		
Part. No.	RP1 (mmHg)	MP1 (mmHg)	PP1 (mmHg)	RP2 (mmHg)	MP2 (mmHg)	PP2 (mmHg)
1	34.0 ± 20.8	72.2 ± 44.4	102.2 ± 59.7	39.7 ± 20.4	92.5 ± 22.5	195.9 ± 110.2
2	37.6 ± 20.7	22.9 ± 15.9	13.8 ± 14.7	27.4 ± 24.4	115.1 ± 66.7	264.0 ± 221.1
3	46.6 ± 12.5	36.7 ± 13.8	31.7 ± 15.0	51.6 ± 18.7	93.1 ± 31.1	127.8 ± 45.8
4	*86.6* ± 29.8	125.1 ± 31.8	154.1 ± 43.2	77.1 ± 30.7	143.4 ± 51.0	215.2 ± 117.6
5	136.1 ± 19.7	182.8 ± 39.5	209.8 ± 55.3	134.7 ± 19.4	188.1 ± 47.3	272.8 ± 101.7
6	47.9 ± 31.2	80.7 ± 25.6	107.4 ± 27.9	34.6 ± 37.1	109.5 ± 41.2	168.6 ± 92.4
7	49.5 ± 11.9	93.9 ± 33.3	134.6 ± 68.8	61.1 ± 24.8	101.3 ± 30.9	146.5 ± 53.9

RP1/RP2: UES RP of P1/P2.
MP1/MP2: MP of P1/P2.
PP1/PP2: PP of P1/P2 (lowest pressure in case of a pressure drop; see P1 in participants no. 2 and 3).
MP = mean pressure; P = pressure phase; PP = peak pressure; RP = resting pressure; UES = upper esophageal sphincter.

TABLE IV.
Individual Mean Value and Standard Deviation for LAR2 Latency Determined by High-Speed Image Data

Participant No.	MV (ms)	SD (ms)
1	211.53	84.7
2	238.20	84.5
3	194.42	89.4
4	221.80	77.2
5	233.47	104.3
6	220.32	83.8
7	324.54	121.6

MV = mean value; SD = standard deviation.

would be helpful in terms of protecting the deeper airways. When coughing is triggered as a result of laryngeal penetration or aspiration, intrathoracic pressure increases, which is then released abruptly by opening of the vocal folds.[35,36] This impulse also affects the esophagus and can promote regurgitation of material. A simultaneous UES contraction would help to avoid regurgitation and thus protect the respiratory tract.[37] The data collected in this study confirm that such a cross-organ protective reflex exists in the UES.

By combining the MIT-LAR technique and simultaneous HRM recordings used previously to monitor UES activity,[14] we were able to analyze UES pressure pre- and post-LAR onset at a very high temporal and spatial resolution. Furthermore, the very small catheter diameter reduced irritation of the UES mucosa, which resulted in less UES pretension and more accurate measurements.[38] This investigative method enabled the discovery of the pressure phases P1 and P2 as separately occurring UES responses following the elicitation of the LAR.

It had been previously reported that the laryngo-UES contractile reflex could be triggered better if the stimulus during the FEESST method was applied longer (2 seconds).[23] With that, it became unclear whether a microdroplet would be able to provoke a similar reaction from the UES. MIT-LAR testing is considered to be a safe method to stimulate the laryngeal mucosa for LAR elicitation purposes.[8,9] Besides this intralaryngeal response mechanism, we were able to verify a reproducible UES response pattern after droplet impact in the present study. This proved that the stimulus intensity of a microdroplet is sufficient. Therefore, the MIT-LAR device was an adequate tool for triggering this extralaryngeal reflex mechanism. In particular, the ability to record the droplet's flight, its impact on the laryngeal mucosa, and the laryngeal muscle contractions provided a comprehensive overview of intralaryngeal activities to the examiner, thus potentially enabling further analysis of the relevant LAR parameters.[29] The impact of a microdroplet yields a very small area of stimulation, which accounts for a high testing accuracy in comparison to air pulses as used in other studies.[22,23] A direct stimulation of the UES inlet, which might cause an unwanted contractile response, could therefore effectively be prevented in the present work.

Limitations

The basic concept of a UES response following laryngeal stimulation caused by microdroplets has been proven. The paradoxical appearance of P1 will need further investigation in order to either identify a physiological background or find an alternative explanation for its presence. A limitation of the measuring results by using a unidirectional manometric catheter cannot be completely excluded, although the high-resolution, small-diameter catheter used here is known to only minimally affect the dynamics of the UES.[39,40] Further limitations are certainly given due to the rather small study population size (n = 7), although experiments were performed repeatedly in each individual. On the technical side, droplet formation stability in MIT-LAR should be enhanced as formation of satellite droplets was observed in this study. Fluid system pressure should be controlled by an autonomous system to further increase usability.[41,42]

CONCLUSION

A reaction to laryngeal penetration or aspiration is probably composed of a complex pattern of individual reflexive mechanisms from multiple organs. The UES contributes to this pattern by performing a contractile reflex, preventing regurgitation of gastric or esophageal contents, for example, when coughing occurs for airway clearance.

ACKNOWLEDGMENT

The authors would like to acknowledge the kind support by the Central Research Devices Service Unit of Hannover Medical School, which enabled image and pressure signal synchronization, a fundamental element of the present study. The authors would also like to thank Professor Dr.-Ing. Tobias Ortmaier and Assistant Professor Dr.-Ing. Lüder Alexander Kahrs for supporting this study and Editage for correction of the English text.

Author Contributions. F.J.M.: acquisition, analysis, and interpretation of data, statistics, drafting of the manuscript. M.J.: study concept and design, supervision of acquisition of data, analysis and interpretation of data, statistics, drafting of the manuscript. J.F.F.: technical support, interpretation of data, revision of the manuscript. S.M.: interpretation of data, revision of the manuscript. M.P.: study concept and design, interpretation of data, revision of the manuscript. F.J.M. and M.J. contributed equally to this manuscript and share first authorship. Open access funding enabled and organized by Projekt DEAL.

BIBLIOGRAPHY

1. Lang IM, Dana N, Medda BK, Shaker R. Mechanisms of airway protection during retching, vomiting, and swallowing. *Am J Physiol Gastrointest Liver Physiol* 2002;283:G529–G536.
2. Daggett A, Logemann J, Rademaker A, Pauloski B. Laryngeal penetration during deglutition in normal subjects of various ages. *Dysphagia* 2006;21: 270–274.
3. Miller S, Jungheim M, Kühn D, Ptok M. Endoscopic video imaging of configuration changes in the larynx and pharynx during selected swallowing techniques. [German]. *HNO* 2013;61:623–626.

4. Kaneoka A, Pisegna JM, Inokuchi H, et al. Relationship between laryngeal sensory deficits, aspiration, and pneumonia in patients with dysphagia. *Dysphagia* 2018;33:192–199.
5. Sasaki CT, Suzuki M. Laryngeal reflexes in cat, dog, and man. *Arch Otolaryngol* 1976;102:400–402.
6. Carey BC, Sulica L, Wu A, Branski R. A novel electrodiagnostic assessment of the laryngeal closure reflex. *Muscle Nerve* 2013;47:432–436.
7. Kim T, Goodhart K, Aviv JE, et al. FEESST: a new bedside endoscopic test of the motor and sensory components of swallowing. *Ann Otol Rhinol Laryngol* 1998;107:378–387.
8. Ptok M, Schroeter S. Characterization of the laryngeal adductor reflex by stimulation with microdroplets impulses (microdroplet impulse testing). [German]. *Laryngorhinootologie* 2016;95:482–489.
9. Ptok M, Schroeter S. Deliberate release of the laryngeal adductor reflex via microdroplet impulses: development of a device. [German]. *HNO* 2016;64: 149–155.
10. Ludlow CL, Van Pelt F, Koda J. Characteristics of late response to superior laryngeal nerve stimulation in humans. *Ann Otol Rhinol Laryngol* 1992; 101:127–134.
11. Téllez MJ, Ulkatan S, Blitzer A, Sinclair CF. Unearthing a consistent bilateral R1 component of the laryngeal adductor reflex in awake humans. *Laryngoscope* 2018;128:2581–2587.
12. Lang IM, Shaker R. An update on the physiology of the components of the upper esophageal sphincter. *Dysphagia* 1994;9:229–232.
13. Brownlow H, Whitmore I, Willan PL. A quantitative study of the histochemical and morphometric characteristics of the human cricopharyngeus muscle. *J Anat* 1989;166:67–75.
14. Jungheim M, Schubert C, Miller S, Ptok M. Normative data of pharyngeal and upper esophageal sphincter high resolution manometry. [German]. *Laryngorhinootologie* 2015;94:601–608.
15. Jacob P, Kahrilas PJ, Herzon G, McLaughlin B. Determinants of upper esophageal sphincter pressure in dogs. *Am J Physiol* 1990;259:G245–G251.
16. Szczesniak MM, Fuentealba SE, Burnett A, Cook IJ. Differential relaxation and contractile responses of the human upper esophageal sphincter mediated by interplay of mucosal and deep mechanoreceptor activation. *Am J Physiol Gastrointest Liver Physiol* 2008;294:G982–G988.
17. Shaker R, Ren J, Xie P, Lang IM, Bardan E, Sui Z. Characterization of the pharyngo-UES contractile reflex in humans. *Am J Physiol Gastrointest Liver Physiol* 1997;273:G854–G858.
18. Lang IM, Medda BK, Shaker R. Mechanisms of reflexes induced by esophageal distension. *Am J Physiol Gastrointest Liver Physiol* 2001;281: G1246–G1263.
19. Babaei A, Dua K, Naini SR, et al. Response of the upper esophageal sphincter to esophageal distension is affected by posture, velocity, volume, and composition of the infusate. *Gastroenterology* 2012;142:734–743.
20. Perera L, Kern M, Hofmann C, et al. Manometric evidence for a phonation induced UES contractile reflex. *Am J Physiol Gastrointest Liver Physiol* 2008;294:G885–G891.
21. Shaker R, Ulualp SO, Kannappan A, Narayannan S, Hofmann C. Identification of laryngo-UES contractile reflex in humans. *Gastroenterology* 1999; 116:A1081.
22. Kawamura O, Easterling C, Rittman T, Hofmann C, Shaker R. Optimal stimulus intensity and reliability of air stimulation technique for elicitation of laryngo-upper esophageal sphincter contractile reflex. *Ann Otol Rhinol Laryngol* 2005;114:223–228.
23. Kawamura O, Easterling C, Aslam M, Rittmann T, Hofmann C, Shaker R. Laryngo-upper esophageal sphincter contractile reflex in humans deteriorates with age. *Gastroenterology* 2004;127:57–64.
24. Jungheim M, Miller S, Ptok M. Methodological aspects of high resolution manometry of the pharynx and upper esophageal sphincter. [German]. *Laryngorhinootologie* 2013;92:158–164.
25. Jungheim M, Busche A, Miller S, Schilling N, Schmidt-Thieme L, Ptok M. Calculation of upper esophageal sphincter restitution time from high resolution manometry data using machine learning. *Physiol Behav* 2016;165: 413–424.
26. Jungheim M, Schubert C, Miller PM. Swallowing function after continuous neuromuscular electrical stimulation of the submandibular region evaluated by high-resolution manometry. *Dysphagia* 2017;32: 501–508.
27. Sinclair CF, Téllez MJ, Ulkatan S. Human laryngeal sensory receptor mapping illuminates the mechanisms of laryngeal adductor reflex control. *Laryngoscope* 2018;128:E365–E370.
28. Clouse RE, Staiano A. Topography of the esophageal peristaltic pressure wave. *Am J Physiol* 1991;261:G677–G684.
29. Shock LA, Gallemore BC, Hinkel CJ, et al. Improving the utility of laryngeal adductor reflex testing: a translational tale of mice and men. *Otolaryngol Head Neck Surg* 2015;153:94–101.
30. Tsujimura T, Suzuki T, Yoshihara M, et al. Involvement of hypoglossal and recurrent laryngeal nerves on swallowing pressure. *J Appl Physiol* 2018; 124:1148–1154.
31. Pinna BR, Herbella FAM, de Biase N, Vaiano TCG, Patti MG. High-resolution manometry evaluation of pressures at the pharyngo-upper esophageal area in patients with oropharyngeal dysphagia due to vagal paralysis. *Dysphagia* 2017;32:657–662.
32. Jacob C, Kahrilas PJ, Logemann JA, Shah V, Ha T. Upper esophageal sphincter opening and modulation during swallowing. *Gastroenterology* 1989;97:1469–1478.
33. Trifan A, Shaker R, Ren J, et al. Inhibition of resting lower esophageal sphincter pressure by pharyngeal water stimulation in humans. *Gastroenterology* 1995;108:441–446.
34. Mu L, Sanders I. Neuromuscular organization of the human upper esophageal sphincter. *Ann Otol Rhinol Laryngol* 1998;107:370–377.
35. Miles A, McFarlane M, Scott S, Hunting A. Cough response to aspiration in thin and thick fluids during FEES in hospitalized inpatients. *Int J Lang Commun Disord* 2018;53:909–918.
36. Pitts T. Airway protective mechanisms. *Lung* 2014;192:27–31.
37. Pitts T, Rose MJ, Mortensen AN, et al. Coordination of cough and swallow: a meta-behavioral response to aspiration. *Respir Physiol Neurobiol* 2013; 189:543–551.
38. Xiang X, Tu L, Zhang X, Xie X, Hou X. Influence of the catheter diameter on the investigation of the esophageal motility through solid-state high-resolution manometry. *Dis Esophagus* 2013;26:661–667.
39. Guiu Hernandez E, Gozdzikowska K, Jones R, Huckabee ML. Comparison of unidirectional and circumferential manometric measures within the pharyngoesophageal segment: an exploratory study. *Eur Arch Otorhinolaryngol* 2018;275:2303–2310.
40. Nativ-Zeltzer N, Logemann JA, Zecker SG, Kahrilas PJ. Pressure topography metrics for high-resolution pharyngeal-esophageal manofluorography-a normative study of younger and older adults. *Neurogastroenterol Motil* 2016;28:721–731.
41. Fast JF, Muley A, Kühn D, et al. Towards microprocessor-based control of droplet parameters for endoscopic laryngeal adductor reflex triggering. *Curr Dir Biomed Eng* 2017;3:239–243.
42. Fast JF, Westermann KA, Laves MH, et al. Droplet applicator module for reproducible and controlled endoscopic laryngeal adductor reflex stimulation. *Biomicrofluidics* 2020;14:044112.