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A Missing Sphincteric Component of the Gastro-Esophageal Junction in Patients with GERD

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Abstract

Background—It was recently shown that the tonic pressure contribution to the high-pressure zone (HPZ) of the esophagogastric segment (EGS) contains contributions from three distinct components, two of which are smooth muscle intrinsic sphincter components, a proximal and a distal component (1).

Aim—To compare the pressure contributions from the three sphincteric components in normal subjects with those in GERD patients.

Methods—A simultaneous endoluminal ultrasound (EUS) and manometry catheter was pulled through the esophago-gastric segment in 15 healthy volunteers and 7 patients with symptomatic GERD, before and after administration of atropine. Pre-atropine (complete muscle tone), postatropine (non-muscarinic muscle tone plus residual muscarinic tone), and subtracted (pure muscarinic muscle tone) pressure contributions to the sphincter were averaged after referencing spatially to the right crural diaphragm (RCd) and the pull-through start position.

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Results—In the normal group the atropine-resistant and atropine-attenuated pressures identified the crural and two smooth muscle sphincteric components respectively. The subtraction pressure curve contained proximal and distal peaks. The proximal component moved with the crural sling between FI and FE and the distal component coincided with the gastric sling-clasp fiber muscle complex. The subtraction curve in the GERD patients contained only a single pressure peak that moved with the crural sphincter, while the distal pressure peak of the intrinsic muscle component, which was previously recognized in the normal subjects, was absent.

Conclusions—We hypothesize that the distal muscarinic smooth muscle pressure component (gastric sling/clasp muscle fiber component) is defective in GERD patients.

Introduction

The gastroesophageal junction contains intrinsic smooth muscle and extrinsic skeletal crural muscles that create a high-pressure zone and regulate the flow of fluid between the esophagus and stomach. In addition to controlling esophageal emptying, these muscles provide an anti-reflux barrier to gastric content and control physiologic air venting (belching) and retrograde ejection of gastric contents (vomiting).

The esophagogastric segment (EGS) high-pressure zone is a complex and dynamic structure that is subjected to different forces including active muscle tone and passive elastic stress that resists hiatal opening, intraluminal pressure driving hiatal opening, and a pressure gradient between gastric and intra-thoracic fluid content that drives reflux after hiatal opening. The relationship between the forces is made more complex because:

1. The components of the gastroesophageal junction high-pressure zone overlap and move in relationship to each other with respiration.
2. The tone within the sphincter components change in time along with intra-abdominal pressure.
3. The sphincters may relax spontaneously either as a transient lower esophageal sphincter relaxation (TLESR's) or with swallowing.
4. The forces that drive reflux events are constantly changing with respiration and change with strain.

Numerous investigators have proposed the existence of various components of the gastroesophageal junction high-pressure zone. Ingelfinger(2) argued for skeletal muscle crural diaphragmatic contraction as important to the antireflux barrier. This crural barrier has been confirmed by a number of other investigators. (3,4,5,6,7)

Code et al.(8) reported an intrinsic smooth muscle intraluminal high-pressure zone (HPZ) in the segment between the esophageal body and the gastric cardia. Partial in vivo suppression of sphincteric pressure in humans by atropine,(9,10,11,12,13,14) and studies in which the HPZ was quantified after removal of the gastro-esophageal segment during oesophagogastricectomy in humans(6) led to the proposal that this second intrinsic sphincteric component overlaps spatially with the crural diaphragm.(15,16) Because no anatomic correlate could be found, it has been necessary to assume a purely physiological circular smooth muscle 'lower esophageal sphincter' (LES) intrinsic to the smooth muscle fibers of the esophagus.(17,18)

Liebermann-Meffert et al.(19,20) measured a thickened wall of smooth muscle at the gastric sling/clasp muscle fiber groups in cadavers. 'Sling' muscle fibers surround the junction of the esophagus and the greater curvature side of the gastric cardia. Opposing these are 'clasp' muscle fibers on the lesser curvature junction. Stein et al.(16) argued for an anatomic

smooth-muscle LES defined by the gastric sling/clasp muscle fiber group at the locally thickened esophago-cardiac junction.

In a recent study, Brasseur et al.(1) reconciled these contrasting models by applying simultaneous ultrasound/manometry with pharmacologic attenuation of the intrinsic muscarinic smooth muscle components of the high pressure zone. By the use of atropine with deep inspiration and expiration, they stabilized the sphincter area allowing the non-muscarinic pressure profile to be subtracted from the entire pressure profile of this area after referencing to the crural sphincter leaving only the muscarinic smooth-muscle sphincter contribution. With these subtraction curves Brasseur et al.(1) showed that the gastroesophageal junction high-pressure zone can be separated into three components: two atropine attenuated intrinsic muscarinic smooth muscle components and an atropine resistant skeletal muscle component[the external crural diaphragm (CD)]. The intrinsic and extrinsic components move relative to one another during inspiration and expiration. The two components separate and the esophagogastric segment (EGS) lengthens during expiration. The proximal component and the crural diaphragm move together in lock step during respiration, while the distal component moves relatively independently. However, it should be noted that the distal component also moves with respiration in the same direction that the proximal component moves, just not as far.

The current study compares and contrasts patients with gastroesophageal reflux disease (GERD) to the normal control subject group previously described by Brasseur et al.(1) using the same methodology to evaluate the gastroesophageal junction high-pressure zone. The purpose of this study was to determine if there were any significant differences in the strength or relative positioning of the three sphincteric components in GERD patients. If so, these differences might point to an anatomic or physiologic abnormality that may underlie the pathophysiology of gastroesophageal reflux disease. The data from the GERD patients in the current study were collected at the same time as the normative data reported in Brasseur et al.(1) therefore the GERD group was compared with the previously presented study of the normal volunteer group.

Materials and Methods

Subjects

Fifteen normal volunteer subjects were evaluated (eight male, seven female, 23–47 years, mean age 34 ± 8.5 years) in the study published previously by Brasseur et al.(1) Seven patients (three male and four female) with GERD (33–66 Years, mean age 45 ± 10.7 years) were evaluated over the same time period with the same procedure as the normal subjects. The GERD patients all complained of heartburn and/or regurgitation, which were relieved with high dose proton pump inhibitors. All subjects gave IRB approved informed consent to take part in the studies, and all subjects were tested in accordance with the policies of the National Institute of Health and Temple University School of Medicine. Exclusion criteria for all subjects included subjects on any medication, which could affect the gastroesophageal junction high-pressure zone. This included prokinetic agents, erythromycin type antibiotics and anticholinergics. The following medical problems were also considered exclusion criteria: abdominal surgery involving the stomach or esophagus, diabetes, scleroderma, achalasia and current pregnancy. In addition normal volunteers were excluded if they used antacids, H2 blockers, proton pump inhibitors, had any gastrointestinal symptoms, conditions and disorders including a history of esophagitis, gastrointestinal symptoms such as abdominal pain, heartburn, reflux, regurgitation, chest pain, difficulty swallowing, pain on swallowing, dysphagia, nausea or vomiting, esophageal motility disorders or non cardiac chest pain. (see Brasseur et al.(1) for more details)

Endoscopy

All study subjects underwent upper endoscopy using a Pentax 2900 video endoscope (Pentax, Orangeburg, NY, USA) using topical oral anesthesia with Cetacaine (Getylite Industries, Pennsauken, NJ, USA), with or without sedation. Subjects found to have a hiatal hernia were excluded from the normal group. Hiatal hernia was not an exclusion criterion in the GERD study group.

Equipment

A custom assembly was constructed which combined a 20 MHz ultrasound (US) transducer (Microvasive, Boston Scientific, Watertown MA) with a water perfused manometry catheter. The manometry catheter consisted of a 3 French angiography catheter with a small side hole port at the same level as the ultrasound transducer, to simultaneously obtain gastroesophageal junction high-pressure zone musculature cross-section images and corresponding intraluminal pressures at the same location. The transducer rotated at 15–30 Hz to provide 360 degree esophageal cross-section imaging with 0.1 mm axial slice thickness and a typical penetration of about 2 cm. Images were recorded on VHS videotape at 30 frames/sec on a Kay Elemetrics swallowing workstation (Kay-Elementrics, NJ) to provide temporal synchronization of the two data sources (Fig. 1).

Procedure and data collection

Prior to insertion of the simultaneous ultrasound and manometry assembly into the proximal stomach, the back of each subject's throat was numbed with Cetacaine and the nose numbed with Lidocaine to reduce discomfort during the catheter's passage through the nostril. An intravenous line was prepared to allow for the later administration of atropine.

Ultrasound images were collected and co-localized with manometric pressure in the 15 healthy volunteer subjects and 7 GERD patients with breath holding under full inspiration (FI) and full expiration (FE) during a machine pull-through of the catheter assembly at 5 mm/s from the stomach into the thoracic esophagus. The subject lay supine with his or her back at approximately a 35-degree angle. Subject movement was minimized during the duration of the study. Ultrasound imaging verified that the initial transducer position was in the proximal stomach at both FI and FE. After ensuring the catheter's position was correct, the catheter was marked at the nares to ensure accurate repositioning of the transducer assembly in the stomach at the pull through start (PTS) reference location.

Sphincteric contributions to pressure were measured with the costal diaphragm in the extreme inferior and superior positions. For maximal inferior positioning, the subject was instructed to inhale as deeply as possible and hold his/her breath during 'full-inspiration' (FI) pull-throughs. For maximal superior positioning, the subject exhaled as far as possible and held his/her breath during 'full-expiration' (FE) pull-throughs. Each pull-through began in the stomach at the pre-marked start location of the transducer (pull-through start position) and ended well into the esophageal body. At least three pull-throughs were recorded for each FI and FE respiratory state. Swallowing was monitored, and if the subject swallowed at any time during the pull-through, the entire pull-through was discarded. Subjects were asked to hold their breath after deep inspiration or exhalation in order to quantify the changes in axial pressure variation associated with changes in alignment of smooth *versus* skeletal muscle sphincteric tone from inferior *versus* superior displacement of the costal diaphragm.

Pull-throughs were repeated after injection and intravenous administration of atropine. For each pull-through the axial locations of the distal margin of the right crus muscle (RCd) were quantified to use as a spatial reference when averaging pressures over the 15 normal and 7 GERD patient subjects. In addition to the RCd, the initiation of the pull-through (PTS)

was also used as a spatial reference to determine absolute displacement of the pressure peaks. For this reason great care was taken to return the catheter assembly to its original position by marking the position of the nares on the catheter with a Sharpie pen. The PTS reference was not used to evaluate shifts between the pre- to post-atropine pull-throughs, as the extended passage of time between data collection led to reference drift. All analysis was carried out with in-house computer software or image pro plus software (Image Pro plus version six, Media Cybernetics, Bethesda, MD).

After collecting data in full inspiration and full expiration, the intrinsic muscarinic smooth muscle contribution to the sphincter was attenuated using a pharmaceutical protocol developed by Mittal et al.(12,13) and Fang et al.(21), based on the previous studies by Dodds et al.(9) and Holloway et al.(10) these studies showed dose-dependent partial suppression of the resting sphincteric pressure by atropine. Following the Mittal et al.(12,13) protocol, an initial bolus of atropine ($15 \mu\text{g kg}^{-1}$) was administered intravenously, followed by continuous intravenous atropine infusion at $4 \mu\text{g kg}^{-1} \text{ hr}^{-1}$ during the remainder of the study. After waiting 30 minutes and determining that there was an appropriate increase in heart rate to assure maximal suppression of muscarinic smooth-muscle tone (approximately 40% or greater over baseline heart rate), the data were collected for the same positions and respiratory states as done in the absence of atropine with three additional assembly pull-throughs.

Each subject had multiple insertions of the transducer assembly into the stomach and subsequent data collection during a constant speed retraction with the pull-through machine. Each of these insertions and retractions is defined as a “pull through.”

Data analysis

The crural sling could be clearly identified on the ultrasound images. The crus muscles impinging on the esophageal wall appear as hypoechoic muscle bundles. The proximal (RCp) and distal (RCd) margins of the crural sling adjacent to the esophageal wall were identified by L.M., and checked independently by Q.D., as the first and last extrinsic crus muscle bundles imaged during each pull-through. The ‘width’ of the crural sling was defined as the axial separation between RCd and RCp. To quantify relative anatomic shifts in crural sling location, both proximal and distal crus locations were used as references. There were no statistical differences in the results when using either reference. Therefore, all results use the distal marker (RCd) as the spatial reference for the crural sling. The spatial excursions of the anatomic crus and the pressure signatures between FI and FE were determined by referencing to the location of the transducer assembly in the stomach at the initiation of each pull-through (PTS). This was done both pre and post atropine. To quantify relative anatomical shifts in crural diaphragm location, the RCd and PTS were used.

From the three (or more) pull-throughs per subject for each case, one pull-through was chosen based on best quality of high frequency ultrasound images for determining the anatomic crural sling spatial references. After data collection, separate gastric baseline pressures were determined by Q.D. for each pull-through by averaging the pressure signal 5–10 s just prior to the start of each pull-through, with cough and other obvious artifacts excluded. All pressures were referenced to time averaged gastric baseline pressure.

Reconstructing the muscarinic smooth-muscle (atropine attenuated) pressure distribution

As described above, the administration of atropine partially attenuates the muscarinic components of smooth muscle tone in the gastro-esophageal high-pressure zone segment. Brasseur et al(1) showed that the atropine-resistant pressure distribution is associated with the skeletal crural sling and that any residual smooth muscle non-muscarinic or myogenic

tonic contributions are relatively minor in comparison. The intrinsic muscarinic smooth muscle contribution to pressure was reconstructed by subtracting the post-atropine pressures from the full pre-atropine pressures after spatial referencing to the RCd. This subtraction process leaves the purely muscarinic contribution to pressure since the pressure profile from the crural diaphragm and any residual intrinsic non-muscarinic pressure is removed in the subtraction process. In this way the full pressure distribution, the atropine-resistant and the atropine attenuated intrinsic muscarinic smooth muscle pressure distributions were obtained. These pressures were averaged relative to the inferior margin of the anatomic crural sling when the costal diaphragm was in its extreme superior (FE) and inferior (FI) positions. As done with the normal subjects in Brasseur et al(1) in the GERD group the individual pressure profiles were linearly interpolated onto a grid with time increment of 1/250 s before ensemble averaging.(Fig-2A and Fig-2B)

Measurement of area under the curve

In order to quantify the pressure contributions from each component of the gastroesophageal junction high-pressure zone, the area under the ensemble averaged pressure curve was measured for the GERD patients and reanalyzed for the normal controls using image pro-plus software. The area under the crural diaphragm (atropine resistant) pressure curve was measured from the beginning of the upslope of the pressure curve to the point where the down slope of the pressure curve crossed the zero pressure baseline. The lower intrinsic muscarinic smooth muscle (atropine attenuated) area under the pressure curve was measured from the beginning of the upslope of the subtraction curve to the first minimum. The upper intrinsic muscarinic smooth muscle (atropine attenuated) area under the pressure curve was measured from the beginning of the upslope of the pressure curve after the first minimum to the tubular esophagus above the high-pressure zone. (Fig.3)

Statistics

All statistical tests were performed using the paired Student's T Test with 95% confidence level and assuming equal variances. Ensemble plots are presented from all 15 normal subjects and all 7 GERD patients. Data analysis included the percent contribution of the area under the curve (AUC) from intrinsic muscarinic smooth muscle pressure profiles and the atropine resistant pressure profiles. The means and SD of these values were reported. The above data were evaluated to determine the intrinsic muscarinic smooth muscle (atropine attenuated) pressure profiles and the crural diaphragm (atropine resistant) contributions to the EGS and to determine the effects of respiration on the position and pressure relationships of these pressure profiles.

Results

Analysis of normal control subjects

As described previously by Brasseur et al(1), all 15 normal volunteer subjects had normal esophageal and gastro-esophageal segment high-pressure zone function. The width of the longitudinal segment of the esophageal wall in contact with the crural sling averaged 2.0–2.3 cm, as measured from ultrasound imaging. The lower margin of the crural sling was displaced by 1.9 cm as the costal diaphragm shifted from its inferior-most (FI) to its superior-most (FE) respiratory positions. The ensemble averaged full pressure profiles of the high-pressure zone were compared with the averaged pressure profiles after administering atropine, in FI and FE. These plots were lined up relative to the distal margin of the RCd. (Fig-3)

In normal volunteer subjects the distal intrinsic muscarinic smooth muscle pressure component contributes 34% of the AUC of the entire pressure profile to the gastroesophageal junction high-pressure zone in FI and 31% in FE (Table 1) (Fig – 3)

Analysis of GERD patients

Two of the GERD patients were found to have hiatal hernia at endoscopy (one small and one moderate sized hiatal hernia). The other five GERD patients showed no sign of hiatal hernia. The pre minus the post atropine subtraction curves in the GERD patients demonstrated distinctly different pressure profiles from the subtraction curves in the normal volunteer subjects in both full inspiration and full expiration (Fig. 3B).

In the GERD patients the subtraction curve, demonstrated that the distal intrinsic muscarinic smooth muscle pressure peak (lower LES), which was present in the normal volunteer subjects, was absent in both the inspiratory and expiratory phases in the GERD patients, while the proximal intrinsic muscarinic smooth muscle pressure peak (upper LES), seen previously in the normal volunteer subjects, was present and at the same axial position relative to the RCd as in the normal volunteer subjects.

In the GERD group, the width of the gastroesophageal junction high-pressure zone during FI was 2.5 ± 0.7 cm and the width during FE was 2.5 ± 0.7 cm ($p = 0.9$). Unlike the normal volunteer subjects, there was no significant change in the width of the high-pressure zone between FI and FE. The width of the CD during FI was 2.1 ± 0.8 and during FE was 2.1 ± 0.6 ($p = .9$). Like the normal control subjects, the width of the CD did not change between FI and FE. These results indicate that there was no lengthening of the gastroesophageal junction high-pressure zone from FI to FE in the GERD patients as opposed to the normal volunteer subjects. The beginning of the RCd moved 1.4 cm proximally relative to the initiation of the pull-through start position between FI and FE; the intrinsic muscarinic smooth muscle pressure profile moved approximately the same distance in concert with the CD. This is also approximately the same distance that the RCd moved between FI and FE in the normal volunteer subjects.

In the GERD patients the distal intrinsic muscarinic smooth muscle pressure component (lower LES) made no contribution to the pressure of the gastroesophageal junction high-pressure zone pressure profile (Table 2).

Discussion

The intrinsic sphincter (esophageal smooth muscle sphincter) and the crural diaphragm (external skeletal muscle sphincter) are anatomically superimposed in normal individuals. The intraluminal pressure is a summation of pressures from all of these muscle groups(1). Distinguishing the components of the distal esophageal high-pressure zone is important because these pressures reflect anatomic and/or physiologic components of the sphincter and because these pressures combine to equal the closure forces that contributes to and maintains the function of the anti-reflux barrier.

The current study compared the gastroesophageal junction high-pressure zone pressure profile in GERD patients to the gastroesophageal junction high-pressure zone pressure profile in normal volunteer subjects. In our previous work in normal control subjects we administered cis-atracurium and completely abolished the skeletal crural muscle without affecting the smooth muscle sphincter. The smooth muscle contribution to the high-pressure zone was measured directly. The post-cis-atracurium pressure distribution displayed the same double-peaked patterns as was seen using the atropine subtraction method in normal volunteers. The fact that the same double-peaked pattern, of approximately the same

magnitude in pressure, was demonstrated with the use of cis-atracurium as in the atropine subtraction curves is strong evidence that the atropine subtraction curves account for most of the muscarinic tone within the two intrinsic esophageal smooth muscle components.

However, when designing the current study we took into account the fact that atropine does not eliminate all of the muscarinic tone. We therefore designed the current study, from the outset, with that fact in mind. When we subtract the pre atropine pressure from the post atropine pressure, the subtraction process eliminates all of the pressure that is due to non-muscarinic tone. Even though atropine does not eliminate all of the muscarinic tone, what is left after the subtraction process, is pure muscarinic tone (pressure). Pressure due to anything non-muscarinic has been subtracted away. When designing the study we also took into account the fact that atropine may have variable effects on different individuals. We therefore used each individual subject as their own internal control pre atropine and subtracted each individual subject's post atropine pressure curve from the pre atropine pressure curve. Thus, we were able to eliminate the problem of the variable effect of atropine between subjects.

Although the manometric technique, that was used in this study, is somewhat limited by having only one pressure port, we believe that it is more than sufficient to prove the main hypothesis of this manuscript, that the distal pressure profile is present in normal control subjects and absent in GERD patients. The manometric analysis was limited by the fact that the investigators had to design and build the technology to perform these studies. Never the less, even though the HPZ is known to be asymmetric, we strongly believe that what we have demonstrated in this study does not need sophisticated vector volume manometry. We demonstrated that the distal intrinsic esophageal muscarinic pressure component is absent in GERD subjects and it is unnecessary to show the symmetry or asymmetry of a pressure profile that is absent.

The three high-pressure zone components in normal subjects consist of a proximal intrinsic muscarinic smooth muscle pressure component (the upper LES), a distal intrinsic muscarinic smooth muscle component (the lower LES) and an atropine resistant pressure component (the external crural diaphragm). Each pressure component was localized spatially with respect to the other pressure components, and the percent contribution of each pressure component to the antireflux barrier gastroesophageal junction high-pressure zone was quantified by measuring the area under the ensemble averaged pressure curves. It was determined that the intrinsic proximal muscarinic smooth muscle component and the crural diaphragm move in lock step with each other during respiration and move away from the distal intrinsic muscarinic smooth muscle pressure component during full expiration, thus accounting for the lengthening of the high pressure zone between full inspiration and full expiration. It should be noted that both the proximal and distal smooth muscle pressure components move in the same direction during respiration, but that the distal component moves less than the proximal component.

The high-pressure zone in GERD patients (Fig. 5A) differs dramatically from the high-pressure zone in normal control subjects (Fig 5B). The distal intrinsic muscarinic smooth muscle pressure profile that was demonstrated in the normal control subjects is absent in GERD patients whether or not a hiatal hernia is present. In the normal volunteer subjects the gastroesophageal junction high-pressure zone pressure profile lengthens due to the proximal intrinsic muscarinic smooth muscle pressure component moving proximally away from the relatively fixed distal intrinsic muscarinic smooth muscle pressure component. While the results in the GERD patients also demonstrate respiratory movement of the intrinsic muscarinic smooth muscle pressure component and the crural diaphragm in lock step, the width of the gastroesophageal junction high-pressure zone pressure profile remained

unchanged between FI and FE. The explanation for this is that there is no distal intrinsic muscarinic smooth muscle pressure component. Therefore when the intrinsic muscarinic smooth muscle pressure component and crural diaphragm move it does not lengthen the high-pressure zone (no distal component to move away from).

In normal volunteer subjects the distal intrinsic muscarinic smooth muscle pressure component contributes 34% of the AUC of the entire pressure profile to the gastroesophageal junction high-pressure zone in FI and 31% in FE (Table 1). In the GERD patients the distal intrinsic muscarinic smooth muscle pressure component (lower LES) made no contribution to the pressure of the gastroesophageal junction high-pressure zone pressure profile (Table 2).(Fig.4)

The proximal intrinsic muscarinic smooth muscle pressure profile appears to be a physiologic sphincter of esophageal circular smooth muscle (1). Given the close correspondence of the pressure contribution defining the upper LES in the normal group with the single pressure contribution in the GERD group, we propose that these two pressure contributions arise from the same intrinsic muscarinic smooth muscle sphincteric component in both normal subjects and GERD patients. We base this conclusion on two observations. First, the location of this intrinsic muscarinic smooth muscle pressure component relative to the RCd in both the normal volunteer subjects and in the GERD patients is the same. Second, this intrinsic muscarinic smooth muscle component moves in lock step with the crural diaphragm during respiration in both the normal subjects and in the GERD patients. Thus, in both the normal and GERD groups the crural diaphragm is rigidly attached to this intrinsic muscarinic smooth muscle pressure component by the phrenoesophageal ligament.

The distal muscarinic pressure peak normally constitutes one third of the gastroesophageal junction high-pressure zone pressure profile as measured by the area under the curve in normal subjects. This pressure profile may be important to the anti reflux barrier, since it is the most distal component at the EGS and therefore the first line of defense against reflux of gastric contents into the esophagus in the resting state. From the normal volunteer data, this distal muscarinic pressure profile complex remains relatively stationary while the crural diaphragm and proximal intrinsic muscarinic smooth muscle pressure components move proximally about 2 cm during FE. Without the distal muscarinic pressure profile, the distal esophagus is unprotected and may be exposed to gastric pressure, increasing the probability of opening during the resting state. It is not clear what causes the loss of pressure of the distal muscarinic muscle fiber complex in GERD patients. However, there are no apparent sonographic abnormalities in this region. Since the loss of this distal pressure profile appears to be present in GERD patients with and without hiatal hernia. The loss of this lower contribution to the sphincter may account for the abnormal pressure profile reconstructed in hiatal hernia patients by Kahrilas et al.(22), as discussed in Brasseur et al.(1)

Anatomical changes of a hiatal hernia may explain the loss of the 3rd distal intrinsic esophageal muscarinic component as the so called Hill valve is effaced. However, the loss of this component in GERD patients without hiatal hernias was also noted. Even if this pressure loss in GERD patients with hiatal hernias is due to effacement of the Hill valve we believe that this loss of pressure is likely an underlying cause of GERD.

The results of this study provide new knowledge and understanding of the anatomy and physiology of the gastroesophageal junction high-pressure zone and insight into the abnormalities associated with GERD. We hypothesize that the distal intrinsic muscarinic pressure profile is due to the gastric sling/clasp fiber muscle complex. We hypothesize that the lack of contribution from the gastric sling/clasp muscle fiber complex at the junction between the esophagus and gastric cardia is an underlying pathophysiologic abnormality

associated with gastroesophageal reflux disease. The underlying cause of the absence of the distal intrinsic muscarinic smooth muscle pressure profile implies either a weak gastric sling/clasp muscle fiber complex, or a distended esophagus/cardia region, or both. If there is a weak sling/clasp muscle fiber complex than this is either due to a myogenic or a neurogenic defect of either the gastric sling muscle fibers, the gastric clasp muscle fibers or both. If there is a distended esophagus/cardia region than this is either due to a hiatal hernia or due to an anatomic (conformational) abnormality such that the gastric clasp muscle fibers do not oppose the gastric sling muscle fibers. Future studies should be directed at determining the etiology and pathophysiology of the absence of the distal intrinsic muscarinic smooth muscle pressure profile.

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References

1. Brasseur JG, Ulerich R, Dai Q, et al. Pharmacological dissection of the human gastroesophageal segment into three sphincteric components. *J Physiol*. 2007; 580(3):961–975. [PubMed: 17289789]
2. Ingelfinger FJ. Esophageal motility. *Physiology Review*. 1958; 38:533–84.
3. Boyle JT, Altschuler SM, Nixon TE, et al. Role of the diaphragm in the genesis of the lower esophageal sphincter pressure in the cat. *Gastroenterology*. 1985; 88:723–30. [PubMed: 3967808]
4. Mittal RK, Rochester DF, McCallum RW. Electrical and mechanical activity in the human lower esophageal sphincter during diaphragmatic contraction. *J Clin Invest*. 1988; 81:1182–1189. [PubMed: 3350968]
5. Martin CJ, Dodds WJ, Liem HH, et al. Diaphragmatic contribution to gastroesophageal competence and reflux in dogs. *Am J Physiol Gastrointest Liver Physiol*. 1992; 263:G551–G557.
6. Klein WA, Parkman HP, Dempsey DT, Fisher RS. Sphincter-like thoracoabdominal high pressure zone after esophagogastricomy. *Gastroenterology*. 1993; 105:1362–1369. [PubMed: 8224640]
7. Peck N, Callander N, Watson A. Manometric assessment of the effect of the diaphragmatic crural sling in gastro-oesophageal reflux: implications for surgical management. *Br J Surg*. 1995; 82:798–801. [PubMed: 7627515]
8. Code CF, Fyke FE Jr, Schlegel JF. The gastroesophageal sphincter in healthy human beings. *Gastroenterologia*. 1956; 86:135–150. [PubMed: 13384582]
9. Dodds WJ, Dent J, Hogan WJ, et al. Effect of atropine on esophageal motor function in humans. *Am J of Physiology*. 1981; 240:G290–G296.
10. Holloway RM, Dodds WJ, Helm JF, et al. Integrity of cholinergic innervation to the lower esophageal sphincter in achalasia. *Gastroenterology*. 1986; 90:924–929. [PubMed: 3949120]
11. Mittal RK, Fisher M, McCallum RW, et al. Human lower esophageal sphincter pressure response to increased intra-abdominal pressure. *Am J Physiol Gastrointest Liver Physiol*. 1990; 258:G624–G630.
12. Mittal RK, Holloway R, Dent J. Effect of atropine on the frequency of reflux and transient lower esophageal sphincter relaxation in normal subject. *Gastroenterology*. 1995; 109:1547–1554. [PubMed: 7557138]
13. Mittal RK, Chiareli C, Liu J, et al. Atropine inhibits gastric distension and pharyngeal receptor mediated lower oesophageal sphincter relaxation. *Gut*. 1997; 41:285–290. [PubMed: 9378379]
14. Fung KP, Math MV, Ho CO, Yap KM. Midazolam as a sedative in esophageal manometry: a study of the effect on esophageal motility. *J Pediatr Gastroenterol Nutr*. 1992; 15:85–88. [PubMed: 1403454]
15. Kahrilas PJ. Anatomy and physiology of the gastroesophageal junction. *Gastroenterol Clin North Am*. 1997; 26:467–486. [PubMed: 9309398]
16. Stein HJ, DeMeester TR, Naspetti R, et al. Three-dimensional imaging of the LES in gastro-esophageal reflux disease. *Ann Surg*. 1991; 214:374–384. [PubMed: 1953093]

17. Christensen J. Pharmacology of esophageal motor function. *Ann Rev Pharmacol.* 1975; 15:243–255. [PubMed: 238461]
18. Christensen, J. Motor functions of the pharynx and esophagus. In: Johnson, LR., editor. *Physiology of the Gastro-Intestinal Tract.* 2nd. Vol. 1. Raven Press; New York: 1987. p. 595-612.
19. Liebermann-Meffert D, Allgöwer M, Schmid P, Blum A. Muscular equivalent of the lower esophageal sphincter. *Gastroenterology.* 1979; 76:31–38. [PubMed: 81791]
20. Stein HJ, Liebermann-Meffert D, DeMeester TR, Siewert JR. Three-dimensional pressure image and muscular structure of the human lower esophageal sphincter. *Surgery.* 1995; 117:692–698. [PubMed: 7778032]
21. Fang JC, Sarosiek I, Yamamoto Y, et al. Cholinergic blockade inhibits gastro-oesophageal sphincter relaxation through a central mechanism. *Gut.* 1999; 44:603–607. [PubMed: 10205193]
22. Kahrilas PJ, Lin S, Chen J, et al. The effect of hiatus hernia on gastro-oesophageal junction pressure. *Gut.* 1999; 44:476–482. [PubMed: 10075953]

Abbreviations

AUC	Area Under the Curve
CD	Crural Diaphragm
EGS	EsophagoGastric Segment
EUS	Endoluminal UltraSound
FI	Full Inspiration
FE	Full Expiration
GERD	Gastro Esophageal Reflux Disease
HPZ	High-Pressure Zone
LES	Lower Esophageal Sphincter
PTS	Pull Through Start
RCd	Right Crural diaphragm Distal
RCp	Right Crural diaphragm Proximal
SD	Standard Deviation
TLESR's	Transient Lower Esophageal Sphincter Relaxation

Fig. 1.

This is a screen of the Kay-Elemetrics workstation showing the ultrasound image and the corresponding manometric pressure (46.0 mmHg). The ultrasound image on the left is shown at the time indicated by the vertical line on the manometry tracing (right). The arrows point to the crural diaphragm.

A custom made pull-through machine provided a calibrated, constant retraction of the simultaneous ultrasound and manometry catheter in a proximal direction through the stomach and the esophagus at 0.5 cm/s. The pressure data were saved to a computer file and the US images digitized into 256 gray levels, 640 × 480 lossless TIFF files.

Fig. 2.

A – This graph shows the full expiratory pressure curve of an individual normal control subject. The red curve is the pre-atropine pressure curve, the green curve is the post atropine pressure curve and the blue curve is the subtraction curve (red curve minus green curve).

The vertical line at zero on the X-axis represents the start of the right crural diaphragm (RCd). Note that there are two pressure peaks in the subtraction curve (blue). The distal peak represents the lower LES and the proximal peak represents the upper LES.

Fig. 2B – This graph shows the full expiratory pressure curve of an individual GERD patient. Note that there is only one pressure peak within the subtraction curve (Blue), which corresponds with the proximal peak in the normal control subjects.

Fig. 3.

Panels 3A and 3B show the ensemble averaged inspiratory and expiratory pressure curves in 15 normal volunteers respectively. The red curve is the preatropine pressure curve in both Full inspiration (FI) and Full expiration (FE). The green curve is the post atropine pressure curve in both FI and FE. The blue curve is the subtraction curve (red curve minus green curve). The vertical line at zero on the X-axis represents the start of the right crural diaphragm (RCd). The box labeled crus represents the extent of the crural diaphragm (CD) on ultrasound imaging. There are two pressure peaks in the subtraction curves in both FI and FE. The upper or more proximal peak reflects an “upper LES” or proximal intrinsic muscarinic component. The distal peak reflects a “lower LES” or distal intrinsic muscarinic component. The area under the pressure curve from the atropine resistant crural diaphragm was measured from the beginning of the upslope to the point where the down slope of the pressure curve crossed the zero pressure baseline (area not shaded in the above graphs). The lower intrinsic muscarinic smooth muscle (atropine attenuated) area under the pressure curve was measured from the beginning of the upslope of the subtraction curve to the first minimum. The upper intrinsic muscarinic smooth muscle area under the pressure curve was measured from the beginning of the upslope of the pressure curve after the first minimum to the tubular esophagus above the high-pressure zone. The area under the graphs of the pressure curves of the GERD patients was performed in a similar manner.

Fig. 4. Panel 4A shows the ensemble averaged pressure curve in full expiration from 7 GERD patients, referenced to RCd. Panel 4B shows the ensemble averaged pressure curve in full expiration from 15 normal control subjects. The red curve is the pre-atropine pressure curve, the green curve is the post atropine pressure curve and the blue curve is the subtraction curve (red curve minus green curve). The vertical line at zero on the X-axis represents the start of the right crural diaphragm (RCd). Note that in the subtraction curve (blue), the proximal intrinsic muscarinic pressure peak is present in the GERD patients and is in the same axial position relative to the RCD as in the normal volunteers as demonstrated by the rose colored vertical line on the right. The subtraction curve between the pre and post atropine pressure curves in GERD patients show that the distal intrinsic muscarinic peak is absent, as demonstrated by the light turquoise colored line and box on the left.

Table 1

Sphincteric contribution in Normal volunteers

	FULL INSPIRATION		FULL EXPIRATION	
	% of total area under the pressure curve	% of intrinsic area under the pressure curve	% of total area under the pressure curve	% of intrinsic area under the pressure curve
Upper LES	25 (14.1)	66 (13.2)	43 (21.7)	69 (26.0)
CD	62 (13.2)	NA	39 (21.5)	NA
Lower LES	13 (7.9)	34 (13.2)	20 (9.1)	31 (26.0)

Table 1 demonstrates the percent contribution of each sphincteric component to the area under the pressure curve.

Table 2

Sphincteric contribution in GERD patients

	FULL INSPIRATION		FULL EXPIRATION	
	% of total area under the pressure curve	% of intrinsic area under the pressure curve	% of total area under the pressure curve	% of intrinsic area under the pressure curve
Upper LES	62.9 (21.1)	100 (13.9)	26 (14.46)	100 (17.2)
CD	37.1 (17.0)	NA	74 (18.8)	NA
Lower LES	0 (0)	0 (0)	0 (0)	0 (0)

Table 2 demonstrates the percent contribution of each sphincteric component to the area under the pressure curve. Note that there is no distal intrinsic muscarinic pressure component in the GERD patients.