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*Gut* 2010 59: 433-440
doi: 10.1136/gut.2009.202606

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Oesophageal shortening: in vivo validation of high-frequency ultrasound measurements of oesophageal muscle wall thickness

Werend Boesmans,1 Pieter Vanden Berghe,1 Ricard Farre,1 Daniel Sifrim1,2

ABSTRACT

Background and aims Assessment of oesophageal muscle wall thickness with high-frequency intraluminal ultrasound (HFIUS) is proposed as a method to evaluate longitudinal muscle contraction and oesophageal shortening in patients with oesophageal symptoms. Studies using this technique suggested that prolonged oesophageal wall thickening can be associated with chest pain and heartburn. Validation studies comparing HFIUS measurements against fluoroscopic investigations of oesophageal shortening are not available. The aim of this study was to evaluate the relationship between oesophageal muscle wall thickening and oesophageal shortening in vivo.

Methods Oesophageal shortening and muscle wall thickness were assessed simultaneously in lightly sedated cats, using fluoroscopic tracking of endoscopically attached metal clips and HFIUS, respectively. Oesophageal shortening was studied during secondary peristalsis and oesophageal mucosal acidification. Video fluoroscopy and HFIUS images were recorded simultaneously and the magnitude and timing of changes in distance between clips and muscle wall thickness were compared.

Results During peristalsis, the distance between the clips was maximally reduced to 33% and the muscle wall thickness was increased to 218% above baseline. Maximal shortening and wall thickening correlated significantly and occurred simultaneously. Likewise, mucosal acidification provoked simultaneous oesophageal shortening (20%) and increased basal muscle wall thickness (40%). Secondary peristalsis during mucosal acidification was associated with strong and prolonged oesophageal shortening.

Conclusions Oesophageal muscle wall thickening, measured with HFIUS is a good predictor of oesophageal shortening and longitudinal muscle contraction. Oesophageal shortening assessed with fluoroscopy coincides with maximal oesophageal muscle wall thickness as measured with HFIUS to assess oesophageal shortening and longitudinal muscle contraction. Oesophageal mucosal acidification provokes basal oesophageal shortening and secondary peristaltic contractions associated with strong and prolonged oesophageal shortening.

INTRODUCTION

The oesophageal muscularis propria consists of a circular muscle layer surrounded by a sheet of longitudinally organised muscle fibres, the longitudinal muscle layer. Contraction of the circular muscle layer causes the lumen to close during oesophageal peristalsis, which can be studied in humans using oesophageal manometry. Contraction of the oesophageal longitudinal muscle results in oesophageal shortening and axial proximal displacement of the lower oesophageal sphincter (LOS). Oesophageal shortening also contributes to efficient bolus transit by concentrating circular muscle fibres in a certain longitudinal segment.1,2 Studying longitudinal muscle function in humans has proven to be difficult due to technical limitations and lack of non-invasive techniques.

High-frequency intraluminal ultrasound (HFIUS) has been suggested as a valid tool to measure longitudinal muscle contraction and oesophageal shortening in human.3–5 Changes in oesophageal muscle wall thickness, monitored by HFIUS, are

Significance of this study

What is already known about this subject?

► Contraction of the oesophageal longitudinal muscle provokes oesophageal shortening during swallowing and transient lower oesophageal sphincter relaxations (tLOSRs).
► Oesophageal shortening can be monitored in humans by tracking mucosal clips using fluoroscopy or with high-resolution manometry.
► It has been proposed that oesophageal longitudinal muscle contraction can be studied using intraluminal HFIUS. Using this technique, investigators have shown that prolonged or exaggerated longitudinal muscle contraction can be associated with symptoms such as dysphagia, chest pain and heartburn.

What are the new findings?

► We demonstrate that maximal oesophageal muscle wall thickness as measured with ultrasound coincides with maximal oesophageal shortening assessed with fluoroscopy.
► This in vivo study validates the use of monitoring oesophageal muscle wall thickness with HFIUS to assess oesophageal shortening and longitudinal muscle contraction.
► Oesophageal mucosal acidification provokes basal oesophageal shortening and secondary peristaltic contractions associated with strong and prolonged oesophageal shortening.

How might it impact on clinical practice in the foreseeable future?

► By confirming the validity of the debated technique used in several previous publications, our results will encourage other investigators to use this technique to study oesophageal motility. It will furthermore stimulate the industry to develop both hardware and software to aid image analysis which will encourage clinicians to use this technique for examining patients with oesophageal symptoms.
Oesophagus

proposed to reflect contraction of the oesophageal longitudinal muscle layer. Over the last decade, several studies using HFIUS shed light on the role of longitudinal muscle contraction in normal oesophageal physiology, pathophysiology of oesophageal motility disorders and gastro-oesophageal reflux disease (GORD). Prolonged longitudinal muscle contraction or sustained oesophageal contraction was shown to be associated with chest pain and/or heartburn. Patients with oesophageal motility disorders and dysphagia have thicker oesophageal muscle layers, and the precise synchrony between contraction of the circular muscle and longitudinal muscle layers is altered in nutcracker oesophagus and eosinophilic oesophagitis. Studies using HFIUS furthermore showed that longitudinal muscle contraction may play an important role in the development of GORD as segmental distal oesophageal shortening has been shown to be an important component of transient LOS relaxations (tLOSRs).

Most of the new concepts about longitudinal muscle contraction in oesophageal pathophysiology come from studies using HFIUS. So far, this technique has only been performed in a few centres and results of studies have been contested due to a lack of published in vivo validation studies. Measuring oesophageal muscle wall thickness using HFIUS is an indirect way to assess longitudinal muscle contraction and relies on the law of mass conservation. It assumes that because fluids cannot be compressed, oesophageal shortening cannot happen without thickening of the longitudinal muscle layer. However, the oesophageal physiology in vivo might not completely fulfil the ideal conditions required by the law of mass conservation. Therefore, in vivo experiments to correlate oesophageal shortening due to longitudinal muscle contraction with simultaneous HFIUS assessments of muscle wall thickness are mandatory to validate this technique and to promote its use for human research and clinical applications.

The aim of the present study was to evaluate the relationship between oesophageal muscle wall thickening (assessed with HFIUS) and oesophageal shortening in vivo during peristalsis and distal oesophageal acidification.

MATERIALS AND METHODS

Animals

Studies were performed on six cats of either sex, weighing between 3 and 5 kg. The animals were fasted overnight and light anaesthesia was induced with ketamine chloride (Parke-Davis, NV Warner-Lambert, Zaventem, Belgium), 10–15 mg/kg intramuscularly for induction and 10 mg/kg intramuscularly every 30 min for maintenance. The ketamine-induced anaesthesia allowed the cats to tolerate intubation while intermittent spontaneous swallowing was preserved. The experiments performed were approved by the Animal Ethics Committee of the KU Leuven.

Measurements of oesophageal shortening using fluoroscopy

Oesophagoscopy was performed with a therapeutic fibreoptic endoscope (Olympus GIF IT10) and the squamocolumnar epithelial junction was identified. With a haemostatic clip-fixing device (Olympus HX-3L), two stainless steel clips were fixed one and five centimetres oral to the squamocolumnar junction. Axial clip movements were tracked using fluoroscopy and calibrated using a radio-opaque ruler.

Measurements of oesophageal muscle wall thickness using HFIUS

The oesophageal muscle wall was imaged using an Ultracross 3.2 30 MHz coronary imaging catheter (3.2F/155 circular muscle) (Boston Scientific, Maple Grove, Minnesota, USA). The catheter was passed through the mouth and endoscopically clipped between the two previously placed metallic clips (see above). In this way muscle wall thickness was measured in a certain segment, avoiding displacement of the catheter during peristaltic events. Additional experiments were performed to examine the need for clipping the HFIUS sensor: the ultrasound sensor was positioned in between the other two clips, allowing free movement of the sensor. Ultrasound images were recorded on a ClearView Ultrasound console (Boston Scientific).

Experimental design

Peristalsis was elicited by rapid infusion of a 5 ml bolus (Gastrografin, Schering, Berlin, Germany). Distal oesophageal acidification was performed using a polyvinyl tube (2 mm diameter) located 1.5 centimetres proximal to the ultrasound sensor. Saline solutions at pH 7.2 and pH 1.0 were infused for 20 min at 2 ml/min on two different days at least 1 week apart.

Analysis

Ultrasound and fluoroscopy video signals were fed into a real-time quad processor video multiplexer (Velleman Components, Vleteren, Belgium) and recorded simultaneously as a single video signal on VHS (PAL). Relevant portions of the recording were sampled (at 10 Hz) and digitised using a framegrabber board (Sensory, Tigard, Oregon, USA) and video recordings of both HFIUS and fluoroscopy were analysed using a set of custom-written routines in Igor Pro (Wavemetrics, Lake Oswego, Oregon, USA). We analysed three parameters: (1) changes in distance between the clips as a measure of shortening (by video fluoroscopy); (2) muscle wall thickness (by HFIUS); and (3) time of luminal occlusion at the HFIUS probe as a measure of circular muscle contraction (by barium video fluoroscopy). To do so, M-mode images or spatiotemporal maps were constructed from the image time series (figure 1). For the ultrasound images a line selection was made perpendicular to the oesophageal wall and the intensity (grey values) of that line for each of the frames was plotted as a vertical line in the M-mode image. The wall thickness then appeared as a black region of a certain width (in millimetres; ordinate) over time (in seconds; abscissa). In this image the inner and outer boundaries were marked semiautomatically and actual width was computed to determine the changes in wall thickness at rest and during peristalsis. Similar to the HFIUS analysis, spatiotemporal maps were constructed from the fluoroscopy recordings. Here the length of the oesophagus was presented as a vertical line and the grey values represent the width of the oesophagus. In these maps, the position of the clips appears as dark artefacts.

The distance between the clips and muscle wall thickness were measured and expressed as percentage change of the initial baseline distance and thickness, respectively. The durations of muscle wall thickening and oesophageal shortening were calculated between the onset (defined as 10% above baseline) and the time point at which the contraction had recovered >90% of its maximal amplitude. The lag time between (onset or maximal) oesophageal shortening and (onset or maximal) muscle wall thickening was also determined and considered positive when shortening preceded thickening.

Statistics

All data are expressed as means (±SEM). Single comparisons were performed by one-sample t tests or unpaired Student t tests when appropriate. The correlation between oesophageal shortening and muscle wall thickening was tested using the
Pearson correlation test. Significance was declared at p<0.05. Statistical analysis was performed with Microsoft Excel 2007 (Microsoft, Redmond, Washington, USA) and GraphPad (GraphPad Software, San Diego, California, USA).

RESULTS

Oesophageal shortening and muscle wall thickening during peristalsis

At rest, the distance between the two endoscopically attached radio-opaque clips in the distal oesophagus was 42.74±1.79 mm and the baseline average muscle wall thickness was 0.50±0.04 mm.

Infusion of the contrast liquid provoked oesophageal distension with increased distance between the two clips of 13.56±4.50% together with a small decrease in muscle wall thickness. Oesophageal distension triggered a secondary peristaltic sequence that was associated with oesophageal shortening and oesophageal muscle wall thickening. Such a motor event propagated the liquid bolus at 7.6±0.4 mm/s. Maximal shortening, as indicated by the minimal distance between the clips, was 33.00±3.02% of baseline (p<0.0001) and the total duration of oesophageal shortening was 11.86±0.88 s. Muscle wall thickness increased to a maximum of 218.73±13.1% above baseline and the total duration of muscle wall thickening lasted for 8.51±0.71 s.

During peristalsis, maximal shortening and maximal muscle wall thickening occurred at the same time (time lag: 0.23±0.15 s; p=0.14) (figure 2) and correlated significantly (r=0.65615, p=0.0017) (figure 3A). Oesophageal shortening onset preceded the increase in muscle wall thickness by 1.95±0.32 s (p=0.0001) and oesophageal shortening lasted longer (11.86±0.88 vs 8.51±0.71 s, p=0.00541).

At the time of maximal shortening and maximal muscle wall thickening, there was complete occlusion of the lumen at the
level of the HFIUS sensor. There was no measurable time interval between complete occlusion of the lumen around the ultrasound sensor and the peak of oesophageal shortening (0.51 ± 0.21 s; p = 0.17). Also the time interval between lumen occlusion and maximal muscle wall thickening was not significant (0.07 ± 0.22 s; p = 0.74).

A separate set of experiments performed without clipping the HFIUS sensor to the mucosa generated similar results. Maximal oesophageal shortening and maximal muscle wall thickening occurred at the same time (time lag: 0.93 ± 0.48 s; p = 0.073). However, when the ultrasound sensor was not clipped, the correlation between maximal oesophageal shortening and maximal muscle wall thickening was no longer significant (r = 0.29119, p = 0.2759).

### Oesophageal shortening and muscle wall thickening during distal oesophageal acidification

After the first 10 min of oesophageal perfusion with a control solution (pH 7.2), there was no oesophageal shortening (0.28 ± 2.4% change in distance between clips compared with preperfusion length). In contrast, after 10 min of mucosal acidification by perfusion of saline at pH 1.0, there was a significant oesophageal shortening (18.7 ± 1.8% change in distance between clips compared with preperfusion length; p = 0.0001) (figure 4C).

Baseline muscle wall thickness remained unaltered after 10 min of oesophageal perfusion with control solution (2.49 ± 3.4% change compared with preperfusion thickness). In contrast, after 10 min of mucosal acidification, there was a significant increase in muscle wall thickness (42.08 ± 3.8% change compared with preperfusion thickness; p < 0.0001) (figure 5C).

Baseline muscle wall thickening and oesophageal shortening during mucosal acidification also correlated significantly (r = 0.69266; p = 0.0042) (figure 3B).

Perfusions with both control and acid solutions elicited secondary peristaltic contractions. As in the previous experiment using rapid bolus infusions, secondary peristalsis induced by slow perfusions was also associated with oesophageal shortening and muscle wall thickening. However, significant differences were observed between effects provoked by control and acid solutions. Secondary peristalsis induced by control solutions provoked maximal oesophageal shortening of 38.9 ± 3.1% and maximal muscle wall thickening of 207.18 ± 13.93%, while oesophageal acidification provoked more pronounced effects on maximal oesophageal shortening (51.1 ± 2.2%, p < 0.01) (figure 4D) and maximal muscle wall thickening (274.43 ± 12.15%, p = 0.001) (figure 5D). Furthermore, the duration of increased muscle wall thickening associated with secondary peristalsis was significantly longer during oesophageal acidification compared with that observed during perfusion of control solution (32.07 ± 3.7 s vs 6.53 ± 0.5 s; p < 0.0001) (figure 5E).

### DISCUSSION

Assessment of oesophageal muscle wall thickness with HFIUS is currently proposed as a method to evaluate oesophageal longitudinal muscle contraction and oesophageal shortening in patients with symptoms such as non-cardiac chest pain, dysphagia and heartburn. Studies using this technique showed that prolonged longitudinal muscle contraction can be associated with chest pain and, more recently, that segmental longitudinal muscle contraction and oesophageal shortening are important components of lower oesophageal shortening relaxations, particularly during tLOSRs.6 7 13 The latter is relevant for the current understanding of the pathophysiology of GORD and for the search for new pharmacological targets to treat GORD. At present,
oesophageal HFIUS is the only technique proposed for clinical assessment of human oesophageal longitudinal muscle function in health and disease. However, in order to bring this technique into widespread clinical application, further validation studies and appropriate analysis software are necessary.

The aim of the present study was to validate this technique by evaluating the relationship between oesophageal muscle wall thickening (assessed with HFIUS) and oesophageal shortening in vivo during peristalsis and distal oesophageal acidification.

The main findings of this study were: (1) during peristalsis, maximal oesophageal shortening (assessed with fluoroscopy) and oesophageal muscle wall thickening (measured using HFIUS) occurred simultaneously and correlated significantly in amplitude; (2) oesophageal mucosal acidification was associated with oesophageal shortening of ~20% and an increase of muscle wall thickness of 40%; and (3) secondary peristalsis evoked during mucosal acidification was associated with strong (67% increase) and prolonged (25 s) muscle wall thickening.

Several techniques have been used to measure oesophageal shortening and/or longitudinal muscle contraction in animals and humans. So far, the following techniques have been used: (1) fluoroscopic tracking of metallic clips, surgically or endoscopically attached to oesophageal mucosa; (2) measurements using strain-gauges surgically attached to the oesophageal serosa; (3) manometric pull-through between the LOS and the upper oesophageal sphincter; and (4) measurements of axial movements of the LOS using high-resolution manometry. Both animal and human studies suggested that oesophageal longitudinal muscle contraction plays a very important role in oesophageal physiology and pathophysiology. Unfortunately, the techniques used in humans are either short lasting (in order to minimise x-ray exposure), invasive (eg, metallic clips) or they provide an indirect estimate of oesophageal shortening (ie, high-resolution manometry as it detects oral movement of the LOS) making it difficult to include functional longitudinal muscle measurements in the clinical evaluation of patients with oesophageal symptoms.

The use of HFIUS to study longitudinal muscle contraction and oesophageal shortening was first proposed by Nicosia and colleagues in 2001. The method is based on the law of conservation of mass that assumes that oesophageal shortening cannot happen without thickening of the longitudinal muscle layer which is apparent as an increase in the muscle cross-sectional area (CSA). In spite of accurate determinations of oesophageal muscle wall thickness and/or muscle CSA in humans, modelling calculations and interpretation of the measurements based on physical laws, in vivo validation studies using simultaneous HFIUS measurements of changes in oesophageal muscle wall thickness and changes in oesophageal length are not available. The time-consuming analysis that was necessary to examine the image sequences has definitely increased.

Figure 4  Effect of intraluminal acid on oesophageal shortening. (A,B) Representative time-lapse images (a, before; b, during; and c, after secondary peristalsis) of feline oesophagus visualised using video fluoroscopy during perfusion with control solution (saline at pH 7.2) (A) or during perfusion with acid (saline at pH 1.0) (B). (C) Baseline distance between the radio-opaque clips (arrows in A and B) decreased after 10 min of acid perfusion compared with perfusion with control solution (***p<0.0001). (D) Distal oesophageal acidification increases oesophageal shortening during secondary peristalsis compared with perfusion with control solution (**p<0.01).
hampered extensive use of this technique and may also underlie the lack of validation studies. We have automated the analysis in an interactive way using a set of programmed routines running in an Igor Pro environment (standard PC or MAC). This analysis module allows the researcher to track markers semiautomatically and indicated the thickness of the walls in a summary M-mode or spatiotemporal map. We predict that with further automation of the software and improved processing speed, analysis times will become negligible.

We performed simultaneous measurements in an animal model evaluating two different conditions: (1) short-lasting longitudinal muscle contractions in the context of oesophageal peristalsis and concomitant phasic circular muscle contractions; and (2) long-lasting longitudinal muscle contraction during...
mucosal acidification in the absence of peristalsis and phasic circular muscle contractions. We found that during peristalsis maximal muscle wall thickening and maximal oesophageal shortening occurred at the same time and that their amplitudes correlate significantly. Also during mucosal acidification muscle wall thickening correlated with oesophageal shortening. These findings validate the use of HFIUS to assess oesophageal muscle wall thickness changes as a measure of longitudinal muscle contraction and oesophageal shortening.

Apart from these main findings, our study also provides some other interesting rather methodological observations. The onset of oesophageal shortening during peristalsis preceded and lasted longer than muscle wall thickening. This was due to inherent differences between both techniques used to assess oesophageal shortening and muscle wall thickness. The timing of changes in distance between the two clips (oesophageal shortening) is affected by segmental shortening of the oesophagus both proximal and distal to the clips, whereas muscle wall thickness was measured at a single point between the clips. Therefore, longitudinal muscle contraction proximal and distal to the HFIUS sensor resulted in oesophageal shortening preceding and outlasting muscle wall thickening at the level of the HFIUS sensor.

Even when the ultrasound sensor was not clipped to the mucosa, maximal shortening and maximal muscle wall thickening occurred at the same time, but during swallowing there was no correlation between their amplitudes. However, during mucosal acidification, the correlation was significant, since shortening is more steady rather than sweep over the sensor. Therefore, in the context of clinical measurements, such a difference might not be relevant provided that the sensor remains located within the oesophageal segment that is evaluated (ie, distal oesophagus or LOS).

During peristalsis, at the time of maximal muscle wall thickening and maximal oesophageal shortening, the oesophageal lumen was completely occluded (as apparent from the fluoroscopy recordings). While we cannot define the timing of maximal circular muscle contraction with our measurements, previous studies demonstrate that peak muscle wall thickening correlates with peak intraluminal pressure as measured with manometry and that luminal occlusion somewhat precedes peak intraluminal pressure. Is circular muscle contraction contributing to maximal oesophageal wall thickening? Based on the law of conservation of mass, previous studies using analysis of oesophageal muscle wall CSA rather than radius measurements (thickness) suggested that an increase in CSA could only be due to longitudinal muscle contraction. We cannot dispute such a suggestion with our measurements. However, the good correlation observed in vivo between our measurements of muscle wall thickening and oesophageal shortening confirms previous suggestions that muscle wall thickness measurements are a good surrogate for oesophageal longitudinal muscle contraction. The measurements of muscle wall radius rather than CSA could also explain the small differences between the results we obtained and those described in earlier studies measuring CSA.

A series of studies by Paterson et al demonstrated that oesophageal mucosal acidification provokes longitudinal muscle contraction and oesophageal shortening. This effect is mediated by activation of mast cells, capsaicin-sensitive afferent neurons and the release of tachykinins. It is possible that such phenomena are initiated by changes in mucosal permeability (dilated intercellular spaces) induced by mucosal acidification. Bhalla et al demonstrated that oesophageal acid perfusion in healthy subjects induces muscle wall thickening and symptoms. Our animal experiments with oesophageal acidification confirmed the previous data and further validate the use of HFIUS to assess oesophageal longitudinal muscle function. Indeed, HFIUS was able to detect long-lasting oesophageal muscle wall thickening in the absence of phasic circular contractions together with oesophageal shortening measured with fluoroscopy. Apart from tonic basal shortening, acidification also provoked long-lasting effects. Secondary peristaltic contractions, evoked by slow intraoesophageal acid perfusion, were associated with very strong and prolonged oesophageal muscle wall thickening and oesophageal shortening. It is possible that secondary peristalsis-associated increased longitudinal muscle contractility during distal oesophageal acidification could provoke symptoms or hypersensitivity in patients with GORD. Current investigations are testing this hypothesis.

Limitations of our study could be: (1) the use of lightly anesthetised cats with effects of anaesthesia on oesophageal motility; (2) the analysis of secondary peristalsis rather than swallow-induced contractions; and (3) the measurement of oesophageal muscle wall thickness rather than CSA. We had to accept the limitations imposed by an in vivo feline model in order to attach several oesophageal clips endoscopically and perform prolonged fluoroscopic exposures. In spite of these technical limitations, we believe that the interpretation of our results could be extrapolated to human conditions.

In summary, we investigated the relationship between oesophageal muscle wall thickness and oesophageal shortening. Oesophageal muscle wall thickness and oesophageal shortening were simultaneously recorded using HFIUS and fluoroscopic tracking of endoscopically placed metallic clips, respectively. We found that oesophageal muscle wall thickening, assessed with HFIUS, is a good predictor of oesophageal longitudinal muscle contraction and oesophageal shortening, both during bolus-induced secondary peristalsis and after distal oesophageal acidification. Tonic oesophageal muscle wall thickening provoked by mucosal acidification can be easily visualised using HFIUS and is apparent as oesophageal shortening, as indicated by the simultaneously performed fluoroscopy recordings.

We conclude that HFIUS is a valid technique to study longitudinal muscle contraction in humans and that HFIUS can be used in clinical investigations in patients with oesophageal symptoms.

Acknowledgements The authors would like to thank Toon De Greef for his excellent technical assistance.

Funding This work is supported by grants from the FWO (Scientific Research Fund, Flanders, Belgium). RF is a postdoctoral fellow of the FWO.

Competing interests None.

Provenance and peer review Not commissioned; externally peer reviewed.

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