The acid pocket, hiatal hernia and TLESRs: essential players in the pathogenesis of gastro-esophageal reflux disease
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Scintigraphic imaging of the acid pocket: an enlarged pocket with acid coating the distal esophagus in GERD patients with hiatal hernia.

Submitted

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Abstract

**Introduction:** The postprandial acid pocket and the influence of a hiatal hernia (HH) on its position may be important determinants of increased acid exposure in GERD patients. To evaluate this hypothesis, we studied the dynamics of the acid pocket and its position relative to the squamocolumnar junction (SCJ) in healthy volunteers (HV) and GERD patients.

**Methods:** 10 HV and 22 GERD patients (12 HH<3cm (s-HH), 10 HH≥3cm (l-HH)) were studied. The margins of the hernia were marked with radio-labelled clips. To visualize the acid pocket, technetium-99m-pertechnetate was injected and images were acquired up to 2h postprandial. In addition, subjects underwent 4-channel pH-metry with pH pull-through at multiple time points.

**Results:** After meal ingestion, clear accumulation of scintigraphic activity in the proximal stomach was observed. The pocket length was significantly enlarged in s-HH and l-HH compared to HV. In 40% of l-HH patients, the acid pocket was located within the HH above the diaphragm, and was associated with radioactive coating of the distal esophagus. The latter was associated with proximal extension of the pH-transition point above the SCJ. In contrast, the pH-transition point was located distal to the SCJ in HV and s-HH. In addition, lower pH values were revealed 2cm above the LES in l-HH during continuous pH measurements.

**Conclusion:** Entrapment of the acid pocket above the diaphragm contributes to the increased risk of acid reflux in large HH patients, and we confirmed the presence of acidic coating of the oesophagus in patients with a pH-transition point above the SCJ.
Introduction

A hiatal hernia (HH) is one of the most important risk factors of gastroesophageal reflux. Patients with a HH have increased acid exposure and increased prevalence of reflux esophagitis caused by impaired refluxate clearance and a weakened gastroesophageal junction.\(^1\)\(^5\) In the presence of a HH, the capacity of the gastroesophageal junction to prevent reflux of gastric contents into the esophagus is hampered, mainly by the anatomical separation of the lower esophageal sphincter (LES) and the crural diaphragm. In addition, previous studies showed that the hiatal sac can function as a reservoir from which ingested fluid can re-reflux into the esophagus during periods of low sphincter pressure.\(^3\)\(^5\) After swallowing a barium bolus, patients with a large HH indeed had impaired clearance with stasis of contrast in the hiatal sac. In a proportion of patients, re-reflux of this contrast even occurred during swallowing. As comparable dynamics will apply when the hiatal sac is filled with gastric contents, this mechanism has been suggested to play a role in the increased acid exposure in patients with gastroesophageal reflux disease (GERD). Evidence to support this hypothesis is however lacking.

Recently, Fletcher et al. demonstrated that gastric acid hardly mixes with ingested food, but instead accumulates into an acid pocket floating on top of the meal.\(^6\) This phenomenon has been forwarded as explanation for the discrepant observation that the pH of the postprandial esophageal refluxate can be lower than the pH in the gastric corpus. Especially as the acid pocket may represent a reservoir from which acid will escape into the esophagus during periods of low LES pressure, subsequent studies have further investigated the potential role of the acid pocket in the pathogenesis of gastroesophageal reflux.\(^7\)\(^9\) Two studies revealed that the acid pocket is larger and extends more proximal in patients with GERD compared to healthy subjects.\(^10\)\(^11\) To what extent these findings explain the increased esophageal acid exposure observed in GERD patients remains however unclear.\(^12\)

In the present study, we hypothesized that the position of the acid pocket relative to the diaphragm, rather than its size, could be an important determinant of acid exposure in the distal esophagus. Especially in patients with a large HH, the acid pocket may be trapped in the hiatal sac above the diaphragm, facilitating the occurrence of gastroesophageal reflux. The position of a HH however changes frequently in time.\(^13\) Therefore, assessment of the acid pocket at a single fixed time point\(^6\)\(^11\) or even at 15 min intervals\(^7\)\(^9\) is insufficient to study the relationship between acid pocket position and gastroesophageal acid exposure. To this end, continuous registration of the acid pocket relative to the crural diaphragm is required. We previously reported preliminary evidence that the acid pocket can be visualized using single photon computed tomography (SPECT) of the stomach.\(^14\) This technique has recently been introduced as a non-invasive tool to assess gastric volume and accommodation, based on the principle that i.v. injected technetium-99m (\(^{99m}\)Tc)-pertechnetate is taken up by the gastric mucosa allowing visualization of the gastric wall.\(^15\)-\(^18\) \(^{99m}\)Tc-pertechnetate, however, largely behaves like chloride ions. Hence, it is secreted by parietal cells together with gastric acid and accumulates into the stomach\(^15\), providing a non-invasive approach to continuously visualize the acid pocket. In the present study we used this mechanism for
dynamic scintigraphic visualization of the postprandial acid pocket in both healthy subjects and GERD patients and assessed the interaction between a HH, the acid pocket and acid exposure.

**Materials and Methods**

**Subjects**

Studies were performed in 12 healthy volunteers (HV) (mean age 32 (range 22-53), 7 men) and 22 GERD patients (mean age 52 (range 19-66), 12 men), of which 12 patients with no or a small hiatal hernia < 3 cm (s-HH) and 10 patients with a large hiatal hernia ≥ 3 cm (l-HH). All HV were free of any gastrointestinal symptoms. Only healthy subjects with a normal upper endoscopy and no HH were included. GERD patients were defined by the presence of esophagitis obtained during a previous upper endoscopy or chronic heartburn symptoms responsive to proton pump inhibitor therapy. None of the subjects had a history of gastrointestinal surgery or took any medication known to influence gastrointestinal motility at time of the study. The use of proton pump inhibitors was discontinued for a minimum of 5 d prior to the study. Intake of alcohol and nicotine was not allowed 12 and 4 h prior to the study, respectively. Each subject gave written informed consent to participate in the study, which was approved by the Medical Ethical Committee of the Academic Medical Center.

**Study design**

All studies were performed after an overnight fast. During upper endoscopy, performed after xylocaine throat spray, both the squamocolumnar junction (SCJ) and the diaphragmatic impression were marked with a 99mTc-pertechnetate-labelled endoscopic clip (Boston Scientific International, Jeffersonville, USA) (Figure 1). After endoscopy, subjects were imaged fluoroscopically to assess the exact size of the hiatal hernia. The position of the clipped diaphragmatic impression and the proximal extent of the gastric folds were checked from liquid barium swallows. The size of the HH was measured using image analysis software and a ruler to correct for magnification. After fluoroscopic evaluation and approximately 90 min after upper endoscopy, the LES was identified by stationary pull-through using a water perfused sleeve manometry catheter. To continuously monitor the acidity in proximal stomach and distal esophagus, a specially designed 4-channel pH catheter (Zinetics 24ME multi-use pH catheter, Medtronic A/S Skovlunde, Denmark) was introduced through an anaesthetised nostril and positioned with the most proximal pH electrode located 2 cm above the proximal margin of the LES, the 2nd electrode 4 cm distal to the proximal pH electrode, ie. within the LES, the 3rd and 4th electrodes were located 6 and 9 cm distal to the proximal pH electrode, respectively (Figure 1). Thereafter, 450 MBq 99mTc-pertechnetate (Ultratechnekow, Mallinckrodt Medical, Petten, The Netherlands) was injected intravenously for visualization of the acid pocket. Thirty min after injection, subjects
received a standardized meal (510 kcal), consisting of 2 pancakes (2x100 g) with jam (2x15 g) and orange juice (200 ml). After the meal, pH-metry and scintigraphic recordings were performed for 2 h. In addition, pH catheter pull-through was performed preprandially and at 15, 30, 45, 60, 90 and 120 min after meal ingestion. All studies were performed in the sitting position, with the subjects leaning backwards against the gamma camera, and their upper body was restrained by a vacuum blanket to minimise movement.

**Recording methods**

**pH recording**

A four-channel antimony pH catheter with external reference was used. Before and after the study the pH electrodes were calibrated at room temperature using pH 1.0 and 7.0 buffer solutions (Medtronic A/S, Skovlunde, Denmark). Calibration at room temperature was corrected by the computer software for pH measurements at body temperature. At the start of each pull-through the pH catheter was further introduced into the stomach until at least the 2 most distal electrodes were measuring pH < 3. The catheter was then withdrawn in 0.5 cm increments every 2 s until the most distal electrode measured esophageal pH (>4). After each pull-through, the catheter was repositioned in its original location. Signals were digitalised, computer-processed, stored and analysed using commercially available software (MMS, Enschede, The Netherlands).
Chapter 4

Scintigraphy
Prior to each endoscopy, two stainless steel clips were radio-labelled by evaporating small drops of $^{99m}$Tc pertechnetate on the curved inside of one jaw with subsequent covering of this jaw with a thin layer of liquid and acid resistant metacylate coating. Finally, approximately 3 MBq $^{99m}$Tc pertechnetate was attached to each clip, remaining fixed under previously tested liquid and acidic conditions. Dynamic scintigraphic images were acquired on a gamma camera system (Orbiter, Siemens Medical Solutions), equipped with a low energy all purpose collimator. Thirty min after intravenous injection of 450 MBq $^{99m}$Tc pertechnetate, a baseline scan was made to identify the marked SCJ and hiatal hernia. Subsequently, after the meal, a dynamic acquisition was made up to 2 h (480 views, 15 s/view, 120min total acquisition time). Every acquisition was processed on a Hermes processing station (Hermes, Nuclear Diagnostics, Stockholm, Sweden) for further analysis.

Data analysis
LES position and length were measured by manometric pull-through, before placement of the pH catheter. The position of the SCJ, considered to be localised 1cm below the upper border of the LES, was calculated for each subject and then averaged, allowing comparisons on pocket position between pull-through and scintigraphy. From the esophagogastroduodenal pH recordings, mean pH values were obtained during 2min intervals every 10min, providing a postprandial pH profile for each pH recording site in the distal esophagus and stomach. Mean pH values were calculated per subject and per group by averaging the values of all healthy subjects, s-HH patients and l-HH patients separately.

During pull-through, the pH step-up point was defined as a persistent increase in pH to esophageal pH above 4. An acid pocket was defined as a drop in pH > 2 units to a pH < 4. For each pocket, its presence, length, location and mean pH were recorded. In addition, gastric pH and the mean pH of the buffering segment were calculated for each pull-through.

The scintigraphic length of the acid pocket and its position relative to the SCJ were measured at 15, 30, 45, 60, 90 and 120 min postprandially. Measurements were made through a straight line in a planar posterior view of the stomach. A pocket was considered as present, when a clear pool of radiolabelled activity was distinguishable in the proximal stomach. In the case of scintigraphic labelling throughout the stomach, no measurements on pocket length were performed. The radiolabelled clips were scintigraphically visualized as clear dots. The distance between the pocket and the SCJ was measured with the centre of the endoscopic clip marking the position of the SCJ.

Statistical analysis
Statistical analysis was performed using SPSS 12.02 software for Windows. Results are presented as mean ± SEM. Comparisons between healthy subjects and GERD patients were made using Student’s t-tests in case of a normal distribution and a Mann-Whitney U test in case of a non-parametric distribution. The correlation between pull-through and SPECT
measurements was determined using the Spearman rank correlation coefficient, performed using Prism software version 4.00 (GraphPad, CA). A P-value of < 0.05 was considered to be significant.

**Results**

**Manometry and fluoroscopy**

In GERD patients, the size of the HH, assessed using fluoroscopy, was 0.9 ± 0.2 cm in the s-HH group, compared to 3.6 ± 0.4 cm in the l-HH patient group. In HV the LES had a mean length of 3.3 ± 0.3 cm (Table 1). In GERD patients, LES length did not differ compared to HV. (Table 1).

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Healthy subjects</th>
<th>s-HH patients</th>
<th>l-HH patients</th>
</tr>
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<tr>
<td>LES length (cm)</td>
<td>3.3 ± 0.3</td>
<td>3.6 ± 0.2</td>
<td>2.8 ± 0.3 §</td>
</tr>
<tr>
<td>length of HH (cm)</td>
<td>-</td>
<td>0.9 ± 0.2</td>
<td>3.6 ± 0.4</td>
</tr>
<tr>
<td>distance between preprandial transition point and SCJ (cm)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>scintigraphy</td>
<td>pull through</td>
<td>scintigraphy</td>
<td>pull through</td>
</tr>
<tr>
<td>min</td>
<td>3.2 ± 0.7</td>
<td>2.4 ± 0.5</td>
<td>2.0 ± 1.1</td>
</tr>
<tr>
<td>max</td>
<td>3.4 ± 0.4</td>
<td>4.4 ± 0.4</td>
<td>5.9 ± 0.5</td>
</tr>
<tr>
<td>mean</td>
<td>3.0 ± 0.1</td>
<td>3.9 ± 0.1 *</td>
<td>5.4 ± 0.2 *</td>
</tr>
<tr>
<td>pocket length (cm)</td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>min</td>
<td>0.3 ± 0.3</td>
<td>1.5 ± 0.6</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>max</td>
<td>1.4 ± 0.4</td>
<td>2.2 ± 0.6</td>
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<tr>
<td>mean</td>
<td>0.8 ± 0.1</td>
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<tr>
<td>pocket pH</td>
<td>1.7 ± 0.1</td>
<td>1.7 ± 0.2</td>
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<tr>
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<td>gastric pH</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.1</td>
</tr>
</tbody>
</table>

* P<0.05 compared to HV; § compared to s-HH but not to HV; # P<0.02 compared to preprandial; a negative value indicates that the pocket extends above the SCJ.

**The acid pocket detected by scintigraphy and pH pull through**

In the fasting state, an acid pocket was detected by pH pull through in 1 HV (8 %) and in 3 GERD patients (14 %). After meal ingestion, pH pull-through showed an acid pocket in 92 % of both HV and s-HH patients and in 80 % of the l-HH patients. The pocket was present from 15-120 min after the meal, with its presence decreasing in time to 33, 67 and 40 % of all HV, s-HH, and l-HH patients, respectively, after 2 h.

Similarly, scintigraphy revealed no acid pocket in HV under fasting conditions, whereas 36 % of the GERD patients showed radiolabelled activity in the proximal stomach before meal
ingestion. This was most often observed in patients with a HH (63%). After meal ingestion, intense accumulation of scintigraphic activity was observed in the proximal stomach in 92% of the HV and in 100% of the GERD patients. This pool of scintigraphic activity could be clearly distinguished from the SCJ and diaphragmatic clips, visualised as a clear dot (Figure 2). One healthy subject showed no distinct pocket, but scintigraphic activity throughout the whole stomach and was excluded from further pocket data analysis. The acid pocket could be visualized during the total postprandial period, from 15-120 min after meal ingestion, with most acid pockets appearing at 15 min after the meal. After approximately 60 min, the acid pocket migrated more distally, most pronounced in HV. Furthermore, as gastric acid secretion increased and was mixed with the ingested food, the radiolabelled region increased in size (Figure 2A).

**Figure 2.** A) Scintigraphic images and concurrent pull through at different time points (15-120 min) of a healthy subject. Radioactive labelled clips are visible at the SCJ. The acid pocket is visible in the fundus. Note that the pocket migrates distally in time and increases in size. From 90 min onwards, the buffering effect of the meal diminishes as observed with pull through. Finally, no distinct pocket can be detected anymore using pull through. B) Scintigraphic image (top panel) and pull through pH recording of a subject with the acid pocket located in the fundus. Although a clear acid pocket is shown by scintigraphy, this was not confirmed with pH pull through. Most likely, the pH catheter bypasses the acid pocket.

**Acid pocket length**

In HV, the mean length of the pocket obtained with pull-through was 2.7 ± 0.2 cm, reaching its maximum of 3.3 ± 0.6 cm at 60 min postprandial (Table 1, Figure 3A). Pocket length was significantly increased in GERD patients compared to HV, especially in patients with a large HH (Table 1, Figure 3).
Scintigraphic imaging of the acid pocket

Figure 3. Pocket length in time in HV (A), s-HH GERD patients (B) and l-HH GERD patients (C), measured with scintigraphy and pull through. Pocket length is significantly increased in patients compared to healthy subjects.

Using scintigraphy, the mean length of the acid pocket in HV was 3.0 ± 0.1 cm, with a minimum of 2.2 ± 0.3 cm at 15 min after the meal, reaching its maximum of 3.4 ± 0.4 cm at 120 min postprandial (Table 1, Figure 3A). Comparable to pH pull through, in GERD patients, the acid pocket length was significantly increased compared to HV, especially in GERD patients with a large HH (Table 1, Figure 3).

Acid pocket position

Before meal intake, the pH-transition point was located distally to the SCJ in all groups. Meal ingestion resulted in a non-significant proximal migration of the transition point in HV. In GERD patients, the transition point significantly migrated proximally, with extension above the SCJ in l-HH patients (Table 1, Figure 4). The postprandial pH-transition point was located above the SCJ throughout the entire study in 16 % of the s-HH patients and in 50 % of the l-HH patients. In the subgroup of l-HH patients with scintigraphic coating of the distal esophagus, the pH-transition point extended 4.0 ± 0.6 cm above the SCJ.

Figure 4. Individual measurements of the pH transition point in relation to the SCJ before the meal and during the entire postprandial period. * P < 0.05 compared to preprandial. § P < 0.05 compared to HV and s-HH patients during the corresponding period.
Scintigraphically, the acid pocket was located immediately distal to the SCJ in most HV (Figure 5). In two HV, however, the pocket was located in the fundus next to the clip positioned at the SCJ. In these subjects, no discernible acid pocket was found during pull-through, most likely as the pH electrode bypassed the pocket (Figure 2B). Comparable to HV, the acid pocket was located just distally to the diaphragm and SCJ in s-HH patients. On the contrary, the acid pocket was extending above the diaphragm during the entire study in 40 % of the l-HH patients. In the rest of the l-HH patients the pocket was largely located distal to the diaphragm but the acid pocket intermittently migrated actively into the hiatal sac in almost all patients (90 %) (Figure 5).

**Figure 5.** A) Representative scintigraphic image of the postprandial acid pocket and SCJ in a healthy subject. The SCJ and the level of the diaphragmatic impression are depicted as a clear dot. B) Image of a s-HH GERD patient with the acid pocket located just distal to the SCJ. C) l-HH GERD patient with the acid pocket located distal to the SCJ, but some acid is trapped in the hiatal sac. D) l-HH patient with the acid pocket completely located within the hiatal sac. E) l-HH patient with a large acid pocket extending into the hiatal sac.

**Esophageal coating**
In those l-HH patients with the acid pocket continuously located within the hiatal sac, we observed frequent episodes of radiolabelling of the distal esophagus (Figure 6). In contrast to the acid pocket, the radiolabelling was less intense, suggesting a thin acid layer coating the esophageal mucosa. If present, the proximal extension of this coating was $5.6 \pm 0.7$ cm above the SCJ.

**Scintigraphy versus pull-through**
The correlation between scintigraphic and pull-through pocket length was $0.73$ ($P < 0.0001$) (Figure 7A). The correlation between the distance between the pocket and SCJ assessed
Figure 6. Image of a l-HH patient with radioactivity in the distal esophagus. No distinct pocket is observed, but intense radiolabelled activity is present throughout the stomach, including part of the hiatal sac. Note the clear, but less intense staining of the distal esophagus. In line with this, pull through did not reveal an acid pocket and the transition point was well located above the SCJ.

Figure 7. Correlation between both techniques, containing data from all groups. A) Relationship between the pocket length measured using scintigraphy vs pull through ($r = 0.73$, $P < 0.0001$). B) Measurements of the distance between the proximal border of the acid pocket and the SCJ. A negative outcome with pull through means the transition point was measured above the SCJ. The patients with coating of the distal esophagus are encircled and located on the left x-axis. Measurements with the possibility of acid covering the pH sensor during pull through are located within the large circle.
using the 2 different techniques was less pronounced \((r = 0.45, P < 0.0001)\) (Figure 7B). This discrepancy was to a large extent observed in l-HH patients; while the pocket was scintigraphically visualised distal to the SCJ, the pH-transition point was located above the SCJ in 50 % of patients. In the majority (90 %) of these patients, additional radiolabelled staining of the distal esophagus was frequently observed. Correlation between the length of the coated segment and the extension of the transition point above the SCJ was significant \((r = 0.95, P < 0.0001)\).

**Acid exposure at the esophagogastric junction**

The pH profile obtained using continuous pH-metry was comparable in HV and s-HH patients, whereas substantial differences were observed in the profile in l-HH patients (Figure 8). Before the meal, the pH measured 2 cm above the LES, within the LES and in the proximal stomach was non-acidic and did not differ between the 3 groups. After meal ingestion, esophageal pH was generally uniform and comparable to preprandial levels in HV and s-HH patients during the 2 h study period. In l-HH patients, however, esophageal pH was decreased and became even acidic at the level of the LES during the first postprandial h. After meal ingestion, the proximal stomach became acidic in all groups, most pronounced in l-HH patients. Note that in l-HH patients the pH sensor measuring proximal stomach pH was located in the distal part of the hiatal sac. Mean pH in the mid/distal stomach

![Figure 8. pH profile of all groups in function of time obtained 2 cm above the LES (A), at the level of the LES (B), in the proximal stomach (C) and in the mid/distal stomach. After meal intake, pH of the most distal esophagus becomes slightly acidic in +HH patients during the first postprandial h. The proximal stomach becomes acidic in all groups after meal intake, reflecting the presence of the acid pocket. In +HH patients, the sensor in the proximal stomach is located within the hiatal sac, remaining acidic throughout the entire study.](image-url)
remained acidic throughout the study in all groups, with the lowest pH measured in l-HH GERD patients.

**Discussion**

In the present study, we introduced a new method for continuous visualization of the acid pocket to evaluate the importance of its position relative to the diaphragm in the pathogenesis of GERD. Our major finding is that in patients with a HH ≥ 3 cm, the acid pocket is enlarged and extends into the hiatal sac above the diaphragm, associated with acidic coating of the distal esophagus in almost half of these patients. In addition, we demonstrated increased postprandial acid exposure in the region of the esophagogastric junction in patients with a large HH, providing evidence that capture of the acid pocket in the HH above the diaphragm contributes to the increased risk of acidic reflux in this subpopulation of GERD patients.

Intravenously injected $^{99m}$Tc-pertechnetate is taken up and secreted by parietal cells in the gastric mucosa and accumulates into the gastric lumen. Using this technique in combination with radiolabelled clipping of the SCJ and diaphragm, we were able to show accumulation of the radiolabel in the proximal stomach, compatible with the acid pocket. Comparison with pull-through data indeed showed a good correlation between both techniques, especially for length and position of the acid pocket. Most importantly, scintigraphy allowed continuous visualization of the pocket throughout the entire study period. Previously, it was questioned whether the acid pocket represents acid floating on top of the meal, or is rather created by a lack of buffering capacity of the meal. In the latter case however, one would rather observe a more diffuse distribution of the radiolabel throughout the stomach and not a clear collection floating on top of the meal. Moreover, in previous studies using SPECT scanning, 3D images clearly showed that the accumulation of scintigraphic activity in the proximal stomach is not located within the stomach wall, but is a reservoir of secreted fluid. Therefore, we are confident that our technique reliably and continuously visualizes the position of the acid pocket.

Before meal intake, no acid pockets were observed in healthy subjects, whereas in 36 % of all GERD patients a preprandial pocket was present, most pronounced in l-HH patients. Previous studies did not reveal any preprandial acid pockets. However, these studies were performed in HV or GERD patients with no or a small HH. Alternatively, although GERD is not considered to be a disease of acid hypersecretion, GERD patients have higher gastric acid secretion than controls, possibly contributing to the presence of an acid pocket during fasting. In contrast, the incidence of acid pockets after meal ingestion was comparable between HV and GERD patients. Maximum pocket length was reached at 60 min after meal ingestion. Interestingly, this time point correlates with the highest rate of acid reflux episodes observed in GERD patients. The size of the acid pocket was significantly increased compared to HV, especially in l-HH patients. Compared to previous studies, the length of the acid pocket measured in our study was larger both in HV
and GERD patients. This difference might be due to methodological differences, as we performed pull through at 0.5 cm intervals and maintained each position for 2 s, whereas others performed pull through at 1 cm intervals every 30 s or 1 min. On the other hand, our scintigraphic measurements correlated well with our pull through recordings. To what extent the increased acid pocket size translates to a larger volume of unbuffered acid, potentially contributing to larger volume and more proximal extending acid reflux, requires further investigation.

More important was our finding that the position of the acid pocket in l-HH patients differed from s-HH patients and HV. In approximately 40% of l-HH patients, the acid pocket is continuously located within the hiatal sac, whereas in the remainder of l-HH patients, acid was intermittently migrating into the hiatal sac throughout the study. This in contrast to the suggestion of Clarke et al. that a hiatal hernia provides a physiological acid pocket as it is lined by acid secreting mucosa. Rather than size, we suggest that this supradiaphragmatic location of the acid pocket represents an important mechanism promoting acid reflux into the distal esophagus. The localization of the LES above the diaphragm has been shown to generate a positive pressure gradient resulting in facilitation of esophagogastric junction opening. Especially in the presence of a large HH, sphincter function is even further impaired. Moreover, as shown in the present study, the acid pocket is entrapped within the hiatal sac above the diaphragm, further creating a combination of conditions promoting reflux of acid material into the esophagus. Indeed, we clearly showed coating of the distal esophagus with radiolabelled material in those patients where the acid pocket was trapped within the hiatal sac. The intensity of this staining was less pronounced compared to the activity of the pocket, most likely compatible with coating of the distal esophageal mucosa with a thin acid layer. This was further confirmed by the observation that the proximal extent of the coating corresponded well with the proximal extension of the pH-transition point above the SCJ. In contrast, Clarke et al. positioned the acid pocket at the level of the SCJ when the acid pocket extended to or above the proximal gastric folds. Therefore, no information on extension of the acid pocket above the SCJ was obtained by that study. Interestingly, our findings are in line with those previously reported by Pandolfino et al. This author suggested that this proximal extension of the transition point above the SCJ most likely represents an acid film coating the mucosa, as it occurred in the context of an intact esophagogastric high pressure zone. In both studies, coating occurred in patients with large hiatal hernias, strengthening the hypothesis that mechanical disruption of the esophagogastric junction promotes either re-reflux from the hiatal sac, poor esophageal clearing or probably diminished buffering capacity of the mucosa or saliva. Here, we actually visualized this acidic coating and confirmed the hypothesis that acid trapped within the hiatal sac significantly contributes to increased acid exposure at the distal esophagus in l-HH patients.

The increase in acid exposure in l-HH patients is also suggested by our pH-metry data at several levels of the gastroesophageal junction. In concordance with previous studies, the pH-transition point before meal intake was located below the SCJ in all subjects and the pH profile in the region of the esophagogastric junction was comparable in all groups.
Comparably with Pandolfino et al.\textsuperscript{11}, meal ingestion resulted in profound differences between patients and HV, with the proximal shift of the pH-transition point extending above the SCJ in I-HH patients. Moreover, continuous pH measurements revealed lower pH values 2 cm above, within and just below the LES in I-HH patients, especially during the first 50 min after the meal. Similarly, Fletcher et al. reported increased acid exposure immediately above the SCJ compared to 5 cm proximal to it.\textsuperscript{23} It should be emphasized though that the pH probe in our study was not clipped to the esophageal wall, and changes in position of the electrodes during the study can not be excluded. Nevertheless, our data further corroborate to the importance of a HH and the position of the acid pocket in the pathogenesis of GERD.

Despite a good correlation between scintigraphy and pH pull-through, some discrepancies occurred between both techniques. First, when the acid pocket is located more towards the fundus, part of the pocket will be easily missed by the pH catheter. Consequently, conventional pH pull-through might incorrectly demonstrate the presence or absence of an acid pocket, whereas scintigraphy provides detailed information on the exact localisation of the pocket. Secondly, as acid contents may be pulled up with the pH sensor during pull-through, the proximal border of the acid pocket may incorrectly be determined more proximally compared to scintigraphy. On the other hand, our scintigraphic method was limited in some cases by the inability to determine the position of the radiolabelled clips because of overlap with the acid pocket. Attempts to label the clips with another radioisotope like indium-111 to overcome this technical problem failed due to limited specific activity available to discriminate the clips. Finally, even when the acid is finally mixed with the meal and buffered, the radioactive label will still be detected and will be misinterpreted as gastric acid, leading to overestimation of pocket length. It could be interesting for future studies to use an alternative radio-labelled meal to locate the meal in relation to the acid pocket and get insight in the mixing properties of the pocket.

In summary, we showed that a supradiaphragmatic position of the acid pocket represents an important mechanism promoting acid reflux into the distal esophagus. Besides a significantly enlarged acid pocket compared to HV, the acid pocket is captured in the hiatal sac in patients with a large HH, leading to increased reflux and acidic coating of the distal esophagus. These findings indicate that entrapment of the acid pocket above the diaphragm contributes to the increased risk of acid reflux in patients with a large hiatal hernia, and confirm the presence of acidic coating of the oesophagus in patients with a pH-transition point above the SCJ.

\textbf{Reference List}


