Original Article

Esophageal Acid Clearance During Random Swallowing Is Faster in Patients with Barrett's Esophagus Than in Healthy Controls

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Background/Aims

Impaired esophageal acid clearance may be a contributing factor in the pathogenesis of Barrett's esophagus. However, few studies have measured acid clearance as such in these patients. In this explorative, cross-sectional study, we aimed to compare esophageal acid clearance and swallowing rate in patients with Barrett's esophagus to that in healthy controls.

Methods

A total of 26 patients with histology-confirmed Barrett's esophagus and 12 healthy controls underwent (1) upper endoscopy, (2) an acid clearance test using a pH-impedance probe under controlled conditions including controlled and random swallowing, and (3) an ambulatory pH-impedance measurement.

Results

Compared with controls and when swallowing randomly, patients cleared acid 46% faster ($P = 0.008$). Furthermore, patients swallowed 60% more frequently (mean swallows/minute: 1.90 ± 0.74 vs 1.19 ± 0.58; $P = 0.005$), and acid clearance time decreased with greater random swallowing rate ($P < 0.001$). Swallowing rate increased with lower distal esophageal baseline impedance ($P = 0.014$). Ambulatory acid exposure was greater in patients ($P = 0.033$), but clearance times assessed from the ambulatory pH-measurement and acid clearance test were not correlated (all $P > 0.3$).

Conclusions

More frequent swallowing and thus faster acid clearance in Barrett's esophagus may constitute a protective reflex due to impaired mucosal integrity and possibly acid hypersensitivity. Despite these reinforced mechanisms, acid clearance ability seems to be overthrown by repeated, retrograde acid reflux, thus resulting in increased esophageal acid exposure and consequently mucosal changes.
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Key Words

Acid clearance; Barrett esophagus; Deglutition; Esophageal pH monitoring; Gastroesophageal reflux

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Introduction

Barrett's esophagus (BE) is a premalignant condition in the esophagus characterized by replacement of squamous epithelium with columnar metaplasia. The prevalence of BE is 1-2%, and its primary clinical relevance lies in the increased risk of esophageal adenocarcinoma.[1] Because of the cancer risk and with considerable healthcare costs, international guidelines recommend surveillance of patients with BE.[2] The main factor underlying the development of BE is believed to be long-lasting gastroesophageal reflux disease.[1] However, although the number and duration of reflux episodes are increased in BE[3]; these patients generally report few symptoms.[4]

Gastroesophageal reflux occurs when reflux contents reach the esophagus by surpassing the barrier at the esophagogastric junction. In this event, esophageal acid clearance in normal subjects usually restores a normal pH within 3-5 minutes.[5] First, esophageal peristalsis clears the majority of the acid ("volume clearance"),[6] and secondly bicarbonate-containing saliva neutralizes any remaining acid by "chemical clearance".[7] Both of these mechanisms are believed to be activated and regulated via reflexes triggered by esophageal acidification and distension.[8,9] When reflux content is not effectively cleared from the esophagus, the remaining acid can cause inflammatory damage to the esophageal mucosa. Over time, these changes can cause the development of erosive esophagitis (EE) or BE, dysplasia, and eventually cancer.[10,11]

A defect in either the afferent (eg, decreased sensitivity) or efferent part (eg, impaired peristalsis or saliva secretion) of the clearance reflex could impair esophageal acid clearance. With respect to the afferent part, this is supported by findings showing hyposensitivity to mechanical distension and heat stimulation in patients with BE.[12,13] Relevant to the efferent part, impaired esophageal motility has been shown to be associated with EE and BE.[14] Several studies have also found increased acid exposure and prolonged acid clearance time in patients with BE using 24-hour esophageal pH-impedance monitoring.[3,15,16] Using impedance measurement, impaired activation of esophageal clearance was recently shown to be associated with dysplastic progression in BE.[17] These findings indicate that acid clearance could be impaired in BE and possibly even be implicated in the development and progression of the condition.

However, since pH-impedance measurements are done under non-standardized circumstances, confounding may arise because the volume, acidity, and extent of the individual reflux episodes are uncontrolled.[18,19] In 1968, Booth et al[20] developed the esophageal acid clearance test, which takes many of these factors into account

by standardizing volume and pH of the bolus to be cleared. Using this test, esophageal acid clearance has been shown to be impaired in patients with EE[20-22] and hiatus hernia (HH),[23,24] but results from the very limited number of studies in patients with BE disagree.[25-27]

We hypothesized that patients with BE have an impaired esophageal acid clearance compared with healthy controls. Using the standardized esophageal acid clearance test, we aimed to (1) measure esophageal acid clearance time (ACT) during several standardized physiologic conditions, (2) compare the swallowing rate in patients with BE and controls, and (3) compare acid clearance test assessment to that of conventional ambulatory pH-impedance monitoring.

## Materials and Methods

Subject Selection

As this was an explorative study and previous data were insufficient to allow this, no sample size calculation was done. However, from previous experience, a sample size of 30 patients with BE and 15 controls was chosen. A post hoc power calculation showed this to be sufficient with an estimated power of 0.85 for the acid clearance time measured during the random swallowing test. A 2:1 ratio was chosen, since the individual variability was expected to be greater in patients. Protocol and written information were approved by the North Denmark Region Committee on Health Research Ethics (project ID: N-20090008) and the study was undertaken in accordance with the Declaration of Helsinki. Patients with BE were recruited as previously described.[28] The inclusion criteria for patients were age between 18 and 80 years and BE defined as intestinal metaplasia in biopsies from salmon-coloured esophageal mucosa $\geq$ 1 cm above the esophagogastric junction.[29] An overview of the patient selection process is shown in Figure 1. Briefly, 937 patients were identified with BE from the pathology database at the Institute of Pathology, Aalborg University Hospital, Aalborg, Denmark. Fourteen healthy controls were recruited among persons, who had previously participated as healthy controls in experiments at our research center. Inclusion criteria for controls were age of 40 years or more, no reflux symptoms at the time of initial screening, and no medication known to affect esophageal sensation. Exclusion criteria were: (1) age below 18 or above 80 years, (2) body mass index (BMI) below 18.5 or above 35 kg/m^2, (3) concomitant disease compromising the subject's safety during participation in the study, (4) significant comorbidity such as alcohol abuse or psychiatric disorders, (5) prior esophageal surgery, and (6) subjects living more

than 100 km from the hospital. For patients, further exclusion criteria were: (1) high-grade dysplasia or cancer in the initial histology report or (2) an endoscopy report stating either a Barrett segment length shorter than C0M2 according to the Prague criteria[30] or a clinical description clearly meaning the same. After exclusions, 89 patients were invited to participate. Of these 33 accepted, but three were later excluded or dropped out, leaving 30 to participate at visit 1. All subjects completed visit 1 with upper endoscopy as previously reported.[28] Four patients were excluded between visit 1 and visit 2

(Fig. 1). Furthermore, one control withdrew consent and another was excluded due to endoscopic signs of EE. Hence, a total of 26 patients and 12 controls remained for inclusion in this part of the study. All clinical experiments in the protocol took place at Aalborg University Hospital, Aalborg, Denmark, from December 2011 to January 2013.

Visit 1: Symptom Assessment and Endoscopy

Figure 2A outlines the individual visits. Before inclusion, all
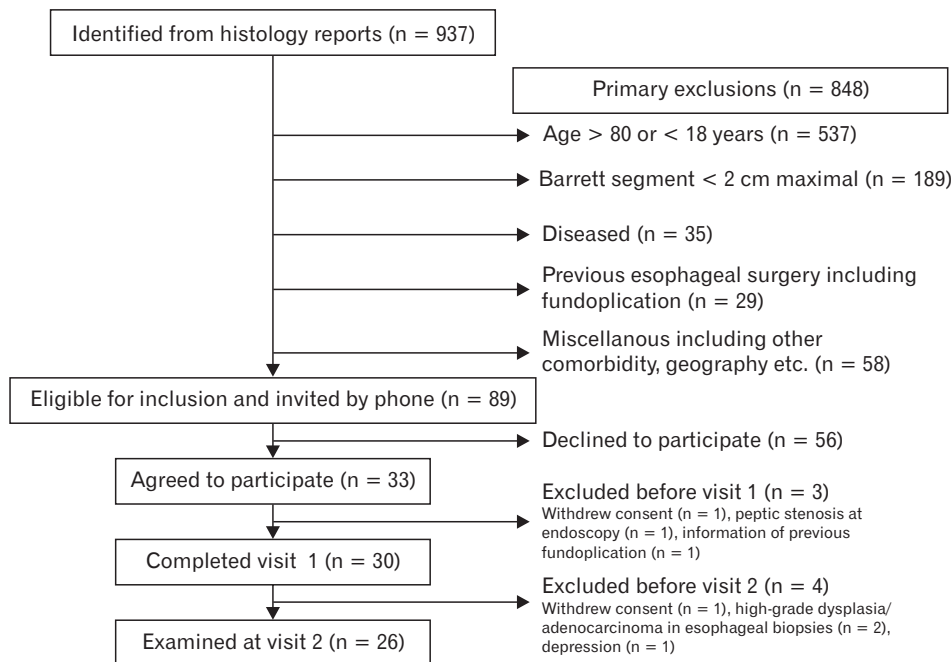
Figure 1. Selection of patients with Barrett's esophagus. A total of 26 patients participated in visit 2. See Figure 2 for details of the individual visits.
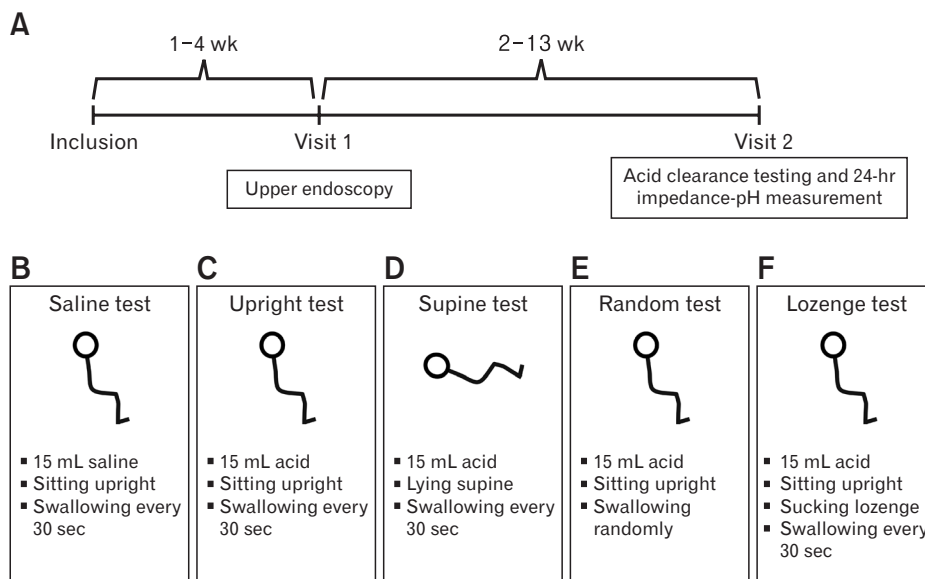
Figure 2. Protocol timeline and the 5 saline and acid clearance tests. (A) Flowchart showing individual visits; all subjects were to complete visit 1 and 2 within 13 weeks; (B) saline clearance test; (C) upright acid clearance test; (D) supine acid clearance test (head-of-bed elevated 30°); (E) random swallowing acid clearance test; and (F) lozenge acid clearance test (sucking a peppermint lozenge).

subjects were informed about the study and gave their written consent to participate. At visit 1, subjects scored frequency of gastrointestinal symptoms on a scale from 0 to 3 (0: less than 1 day per month or not present at all; 1: between 1 day per month and 1 day per week; 2: several days weekly; 3: daily). Grade 1 symptoms on this scale were allowed in controls due to the frequent reporting of reflux symptoms in the general population of this age.[31] After an overnight fast and in all subjects (patients with BE and healthy controls), endoscopies were performed by 2 expert endoscopists using a high-definition endoscope. During the endoscopy, the location of the esophagogastric junction and the crural diaphragm measured from the incisor teeth along with Barrett segment length according to the Prague classification were assessed.[30] A standard biopsy-based urease test of *Helicobacter pylori* (ProntoDry; MIC France, Brignais, France) was performed. To balance sensitivity of the test against patient discomfort and protocol compliance, a 4-day pause in proton pump inhibitor (PPI) treatment before visit 1 was chosen. This time frame has been shown to be sufficient to avoid false-negative results due to PPI treatment.[32] Furthermore, one antrum as well as one corpus biopsy were taken from the stomach, since this increases sensitivity of the test compared with antral biopsies alone.[33]

Visit 2: Acid Clearance Testing and Ambulatory pH Measurement

Acid clearance equipment

A minimum of 2 weeks and a maximum of 13 weeks after visit 1, 26 patients and 12 controls participated in visit 2, before which PPI treatment was also paused for 4 days. Here, the standardized acid clearance test was performed using esophageal acid instillation as previously described[7,34] with minor modifications. The K6011-EI-0632 catheter (Unisensor, Attikon, Switzerland) was used, incorporating 6 impedance channels and 1 ISFET (ion sensitive field effect transistor) pH electrode for high accuracy.[35] The catheter was modified by attaching a pediatric feeding tube for acid instillation. A digital data logger (Ohmega R; MMS B.V., Enschede, the Netherlands) was connected to the catheter. A computer showed pH and impedance live and recorded data. For recording and raw data analysis, MMS Database software (MMS B.V.) was used.

Catheter calibration and placement

The catheter was calibrated using buffers of pH 4.01 and 7.01 as described by the manufacturer and then placed in the esophagus of the fasting subject. Preferably, subjects were intubated nasally, but some could not tolerate this and were intubated orally (Table 1).

For orally intubated subjects, the location of the lower esophageal sphincter (LES) was defined as the endoscopic distance from the incisor teeth to 1 cm above the proximal end of the gastric folds ($Distance_{LES, oral}$).[36] For the subjects intubated nasally, the distance from the incisor teeth to the LES was calculated as [$Distance_{LES, nasal} = Distance_{LES, oral} + 4$ cm].[36] Using these distances, the catheter was then placed with the pH electrode 5 cm, the acid side channel opening 15 cm, and the impedance channels 17, 15, 9, 7, 5, and 3 cm above the LES.

Acid clearance testing

After adjustment to the catheter, mucosal baseline impedance was measured for 5 minutes. Then, 5 different acid clearance tests were performed (Fig. 2B-F). In all tests, isotonic saline 9 mg/mL or 0.1 mol/L hydrochloric acid was instilled rapidly through the side channel using a syringe (duration: 5 seconds). During the tests, subjects swallowed either under controlled conditions every 30 seconds (4 tests) or randomly depending on the subject's natural desire (1 test). From the time of instillation, recordings were made for 15 minutes. In the following order, tests included:

(1) Saline test: 15 mL saline and swallowing controlled
(2) Upright test: 15 mL acid in the upright position and swallowing controlled
(3) Supine test: 15 mL acid in the supine position and swallowing controlled
(4) Random test: 15 mL acid in the upright position and swallowing randomly
(5) Lozenge test: 15 mL acid in the upright position and swallowing controlled, but throughout the whole test sucking an oral peppermint lozenge (Polo; Nestlé UK Ltd, York, UK) to stimulate salivary secretion[37]

The saline test was used as placebo control of acid sensitivity, hence during all tests the subject was blinded to whether saline or acid was used. If pH was still below 4 after 15 minutes (900 seconds), the test was finished anyway and the ACT recorded as 900 seconds. Before the next test, it was ascertained that pH had risen above 4. During the lozenge test, the subject was given one or more new peppermint lozenge(s) when finishing the previous one to ensure constant saliva stimulation. During the entire experiment, the subject registered the following by pressing dedicated buttons on the data logger: planned swallows, random swallows, and the presence and duration of heartburn defined as a subjective sensation of burning in the retrosternal area. If heartburn was present, the subject was asked to score the maximal intensity on a 200 mm scale immediately after each test.[38]

Table 1. Clinical, Endoscopic, and pH-impedance Parameters

	Barrett	Controls	P-value
Demography			
Number of subjects	25[a]	12	NA
Age (yr)	63.9 ± 7.3	54.9 ± 10.8	0.005
Male:female ratio	22:3	8:4	NS
Weight (kg)	84.7 ± 14.4	74.5 ± 10.4	0.036
BMI (kg/m^2)	27.5 ± 3.9	24.2 ± 1.9	0.009
Overweight (body mass index > 25 kg/m^2)	16 (64%)	5 (42%)	NS
Present smoker	7 (28%)	2 (17%)	NS
Alcohol consumption (drinks per wk)	8.0 ± 8.4	5.4 ± 4.3	NS
Proton pump inhibitor use	21 (84%)	0 (0%)	< 0.001
Diabetes mellitus type 2[b]	3 (12%)	0 (0%)	NS
Symptoms			
Heartburn	11 (44%)	1 (8%)	0.058
Regurgitation	9 (36%)	0 (0%)	0.018
Neither heartburn nor regurgitation	12 (48%)	11 (92%)	0.013
Endoscopical data			
Erosive esophagitis present[c]	8 (32%)	NA	NA
Barrett segment circular length[d,f] (cm)	4 (1-18)	NA	NA
Barrett segment maximal length[d,f] (cm)	6 (2-19)	NA	NA
Long-segment Barrett	23 (92%)	0 (0%)	< 0.001
Hiatus hernia present	19 (76%)	1 (8%)	< 0.001
Hiatus hernia length[f] (cm)	3 (0-6)	0 (0-3)	< 0.001
Positive *H. Pylori* test (n)	3 (12%)	1 (8%)	NS
Ambulatory pH-impedance measurement			
Accepted measurement	21 (84%)	10 (83%)	NS
Valid data[e]	18 (72%)	6 (50%)	NS
Duration[f] (hr)	24 (19-26)	24 (15-31)	NS
Total acid exposure time[f] (% time pH < 4)	18 (0-74)	5 (2-16)	0.033
Upright acid exposure time[f] (% time pH < 4)	18 (0-63)	8 (2-23)	NS
Supine acid exposure time[f] (% time pH < 4)	16 (0-88)	0 (0-9)	0.005
Total acid reflux episodes[f] (n)	83 (1-235)	41 (11-59)	NS
Upright acid reflux episodes[f] (n)	61 (1-175)	41 (10-59)	NS
Supine acid reflux episodes[f] (n)	9 (0-98)	0 (0-2)	0.002
DeMeester score[f]	58 (0-215)	15 (5-43)	0.036
Total acid clearance time[f] (sec)	102 (12-264)	96 (54-222)	NS

[a]After exclusion of 1 patient due to excess swallowing.
[b]Orally treated diabetes only.
[c]Grade A or more according to the Los Angeles classification.
[d]According to Prague criteria.[29]
[e]Three patients and 4 controls excluded due to catheter discomfort or technical failure.
[f]Non-normally distributed data.
H. pylori, *Helicobacter pylori*; NA, not applicable; NS, non-significant.
Data shown as mean ± SD, median (range), or proportions.

Ambulatory 24-hour esophageal pH and impedance measurement

Just after the Acid Clearance Test, subjects were asked to participate in an ambulatory pH-impedance measurement using the same catheter. If placed nasally, the catheter was left in place and otherwise relocated to nasal intubation. Following transfer of the clearance test data from the data logger to the computer, a new recording was started and subjects were instructed to record position (upright/supine), meals, and symptoms on the data logger as well

as in a diary. Besides pausing PPI treatment and refraining from acidic beverages during the measurement, no specific restraints were given. Upon returning the assembly, the catheter was post-calibrated followed by data transfer.

Data analysis

Data were blinded for raw data analysis. Recordings were checked for quality, and artifacts were excluded from analysis. Meals were excluded from the ambulatory measurement. ACT was analyzed as the time from drop in esophageal pH below 4 to restoration of pH above 4 (Fig. 3).[7,34] Since to our knowledge no previous publications have defined the time frame for which pH has to be above 4 before acid clearance is considered complete, we based our definition on pilot experiments in healthy controls. Based on these a time period of 15 seconds was chosen, since this gave clearance times similar to previous data.[7,34] Bolus clearance time (BCT) was analyzed manually and defined as the time from a drop in impedance to below 50% of the pre-infusion value to restoration above 50% of the same value for at least 5 seconds.[34,39] Since volume clearance is defined as the first part of the clearance process and chemical clearance as the second, chemical clearance time (CCT) was calculated as $[CCT = ACT - BCT]$.[5] The swallowing rate (swallows/min) during the random test was calculated as: [number of random swallows before reaching pH above 4/random ACT (minutes)].

Statistical Methods

Statistical analyses were performed using Stata 12 (StataCorp LP, College Station, Texas, USA) and with assistance from the Department of Statistics, Aalborg University Hospital. Normally distributed data are expressed as mean ± SD and non-normally distributed data as median (range). In Tables 1 and 2, the distribution of the individual parameters including log transformation is indicated. For comparison between groups of continuous data, the Student's t test, mixed effects analysis of variance (ANOVA), or Kruskall-Wallis' rank sum test were used as appropriate. The Holm-Sidak method was used for post-hoc analysis to adjust for multiple comparisons whenever relevant. For comparison of categorical outcome, Fisher's exact test was used. Relevant cofactors as described in the results were analyzed individually to check for possible confounding.

Swallowing rate is known to affect acid clearance.[40] Therefore, the acid clearance test using random swallowing and those using controlled swallowing were analyzed in separate ANOVAs. Hence, random swallowing rate and clearance times were compared group-wise using one-way ANOVA. In the controlled swallowing tests, clearance times were analyzed as continuous variables using two-way ANOVA with fixed effects parameters being group and the three separate tests (upright, supine, and lozenge).

Results

Baseline Characteristics and Methodology

Demography, endoscopy findings, and ambulatory results are presented in Table 1. At baseline, 44% of patients reported heartburn and 36% regurgitation at least 1 day/month. A hiatus hernia with an axial length of at least 2 cm[41] was present on endoscopy in 76% of patients and 8% of controls ($P < 0.001$), and 92% of patients had long-segment Barrett as defined by at least C0M3.[30] For the acid clearance test, 40% of patients with BE and 42% of controls were nasally intubated (Table 2). One patient was excluded
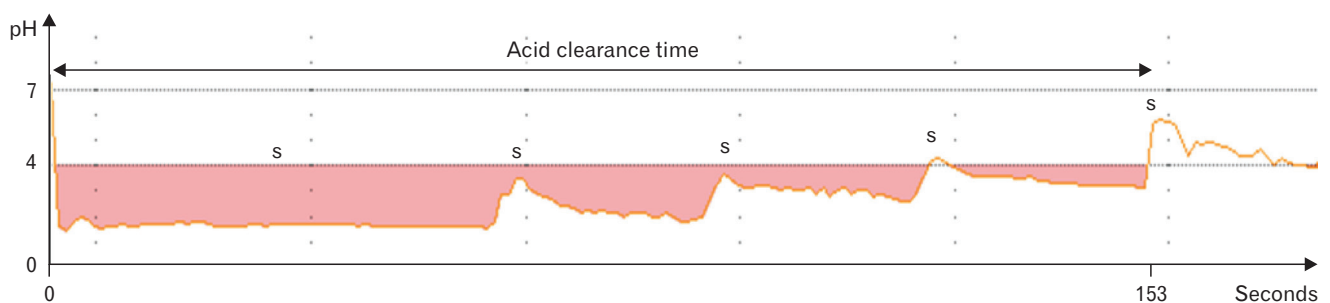
Figure 3. Recording from an upright acid clearance test in a control. The x-axis shows time, the y-axis shows pH value. The pH below 4 is indicated by pink color. Acid is instilled at t = 0 seconds. Swallows are indicated by "**s**". The subject is swallowing every 30 seconds and except for the first swallow, each swallow results in a distinct rise in pH. The acid clearance time is measured as the time from acid instillation until pH is restored above 4. In this case, 5 swallows or 153 seconds were required for this.

from the analysis of random ACT due to poor data quality. Another patient was excluded from the whole data set as an outlier because of a swallowing frequency 5 SDs above the mean during the random swallowing test. In total, 5 acid clearance tests in patients and two in controls were censored at 900 seconds. No acid clearance tests had to be stopped because of discomfort, but 18% of patients with BE and 50% of controls refused to undergo or did not tolerate the ambulatory measurement (Table 1). To some degree, low baseline impedance in patients impeded assessment of BCT during the acid

clearance test, but still data from 72% of patients were available for analysis (Table 2). However, data quality and the same low impedance, combined with dropouts from the ambulatory impedance measurement did not allow sufficient analysis of impedance events. Thus, analysis of the post-reflux swallow-induced peristaltic wave index, as has been done in short-segment BE, was not possible.[17] In fact, some have even suggested refraining from impedance analysis in BE.[42] During the 24-hour pH measurement, patients with BE generally had longer acid exposure time and more acid reflux epi-

Table 2. Results of Acid Clearance Test

	Barrett (n = 25)	Controls (n = 12)		Barrett (n = 25)	Controls (n = 12)
Intubation way			Random acid clearance test		
Nasal	10 (40%)	5 (42%)	ACT[b] (sec)	249 ± 179[e,f]	440 ± 269[f]
Oral	15 (60%)	7 (58%)	BCT[b,c] (sec)	84 ± 69	186 ± 196
Baseline impedance			CCT[b,c] (sec)	158 ± 121	267 ± 170
7 cm above the LES	1360 ± 610	1912 ± 523	Heartburn present (%)	5 (20%)	0 (0%)
5 cm above the LES	1089 ± 472[e]	2061 ± 631	Heartburn duration[d] (sec)	0 (0-180)	0 (0-0)
5 cm above the LES (squamous only[a])	1339 ± 312[e]	2061 ± 631	I-score[d] (mm)	0 (0-148)	0 (0-0)
3 cm above the LES	931 ± 495[e]	2101 ± 782	No of random swallows during test	6.8 ± 5.3	6.5 ± 4.0
Saline test			Swallowing rate (min^{-1})	1.9 ± 0.7[e]	1.2 ± 0.6
BCT[b,c] (sec)	32 ± 19	36 ± 22	Lozenge acid clearance test		
Heartburn present	0 (0%)	0 (0%)	ACT[b] (sec)	177 ± 122[f]	174 ± 91[f]
Heartburn duration[d] (sec)	0 (0-0)	0 (0-0)	BCT[b,c] (sec)	51 ± 35	54 ± 31
I-score[d] (mm)	0 (0-0)	0 (0-0)	CCT[b,c] (sec)	149 ± 119[f]	128 ± 99[f]
Random swallows[d]	0 (0-1)	0 (0-1)	Heartburn present (%)	3 (12%)	1 (8%)
Upright acid clearance test			Heartburn duration[d] (sec)	0 (0-240)	0 (0-60)
ACT[b] (sec)	285 ± 216	219 (88)	I-score[d] (mm)	0 (0-52)	0 (0-0)
BCT[b,c] (sec)	64 ± 42	44 (28)	Non-planned swallows[d]	0 (0-2)	0 (0-2)
CCT[b,c] (sec)	144 ± 121	185 (81)	All tests in total		
Heartburn present (%)	12	0	Heartburn present (%)	8 (32%)	1 (8%)
Heartburn duration[d] (sec)	0 (0-720)	0 (0-0)	Heartburn duration[d] (sec)	0 (0-720)[g]	0 (0-0)
I-score[d] (mm)	0 (0-60)	0 (0-0)	I-score[d] (mm)	0 (0-314)[g]	0 (0-0)
Non-planned swallows[d]	0 (0-2)	0 (0-0)	Non-planned swallows, controlled trials[d] (n)	0 (0-4)	0 (0-3)
Supine acid clearance test					
ACT[b] (sec)	412 ± 269[f]	336 ± 185[f]			
BCT[b,c] (sec)	105 ± 151[f]	78 ± 54[f]			
CCT[b,c] (sec)	265 ± 218[f]	279 ± 168[f]			
Heartburn present (%)	4 (16%)	1 (8%)			
Heartburn duration[d] (sec)	0 (0-180)	0 (0-0)			
I-score[d] (mm)	0 (0-166)	0 (0-0)			
Non-planned swallows[d]	0 (0-3)	0 (0-1)			

[a]Excluding patients with a Barrett segment reaching the electrodes, thus analyzing squamous epithelium data only.
[b]Data were log (ln) transformed for the statistical analysis, but mean and SD shown here are based on raw data.
[c]Data quality only allowed measurement of BCT (and thus calculation of CCT) for 18 patients (72%) and 11 controls (92%).
[d]Non-normally distributed data.
[e]$P < 0.01$ vs controls.
[f]$P < 0.05$ vs upright trial when considering both groups.
[g]$P < 0.05$ vs controls.
LES, lower esophageal sphincter; BCT, bolus clearance time; ACT, acid clearance time; CCT, chemical clearance time; I-score, intensity score of heartburn.
Data shown as mean ± SD, median (range), or proportions depending on distribution.

sodes (Table 1).

Analysis of the cofactors age, sex, BMI, smoking status, and diabetes for possible confounding showed no indication of this. For intubation way and EE presence, some analyses indicated possible confounding. Tentative exclusion of subjects with the highest BMI and age was also performed, thus achieving similar values in the 2 groups. However, neither the performed tentative exclusions nor the adjustment for intubation way and EE presence in the models changed the significance of results. In general as well as in Table 2, unadjusted results and significance are reported.

Barrett's Esophagus Patients Cleared Acid Faster Due to More Frequent Swallowing

Figure 4 and Table 2 show acid clearance test data. Distal esophageal baseline impedance was lower in patients, even when only considering data from squamous epithelium (Table 2). When swallowing randomly, patients swallowed 60% more often (mean swallowing rate 1.9 ± 0.7 vs 1.2 ± 0.6 swallows/min, $P = 0.004$; Fig. 4A) and had a 46% shorter ACT than controls ($P = 0.008$, Fig. 4B). Furthermore, a shorter ACT was correlated with a higher swallowing rate when swallowing randomly ($P < 0.001$, Fig. 4C). When removing the possibility to swallow at will by controlling the swallowing rate however, no differences in clearance times between patients and controls were observed (Table 2). The total duration of heartburn was longer and the scored intensity was stronger in patients than in controls during the acid clearance test (Table 2).

Neither acid clearance time nor acid exposure recorded during the ambulatory pH-impedance measurement could confirm the re-sults of the standardized acid clearance test. Both of these ambulatory parameters showed no correlation with any of the experimental tests (all $P > 0.3$).

Patients with BE with concomitant EE showed a trend to a 64% longer ACT during random swallowing than patients without EE ($P = 0.073$). Accordingly, tentative exclusion of patients with BE also having EE (n = 8) only strengthened the findings, since patients with BE then showed a 54% shorter acid clearance time than controls ($P = 0.003$). As for the controlled swallowing tests, similar stratified analysis and tentative exclusion showed practically no effect of EE presence on acid clearance times (all $P > 0.3$, detailed data not shown). When considering all subjects (patients with BE and controls), ACT during random swallowing was 49% shorter in subjects with an HH than in those without ($P = 0.001$).
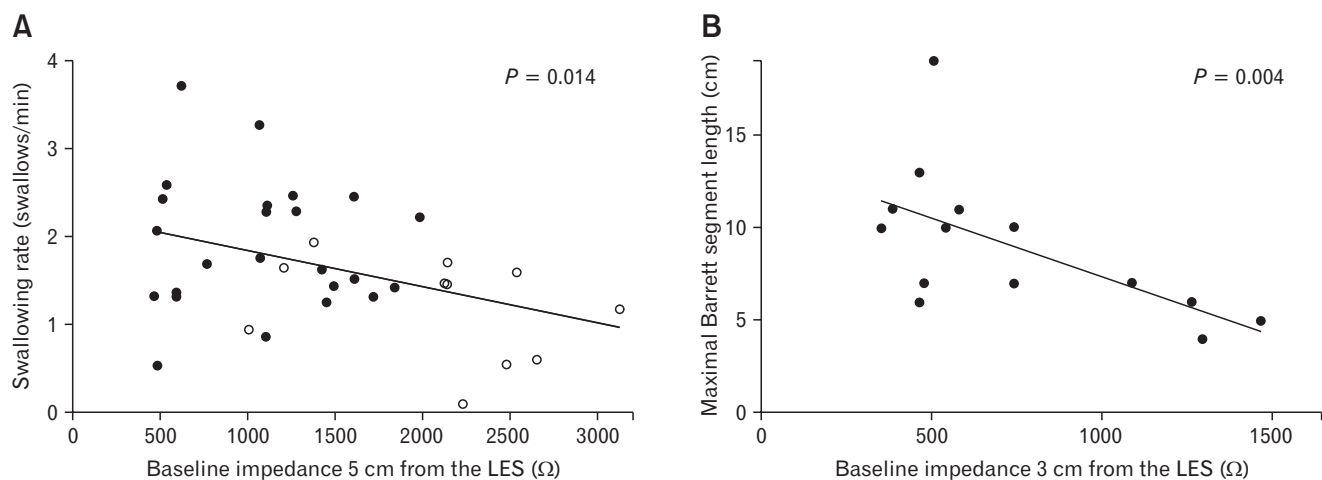
Associations Between Swallowing Rate, Baseline Impedance, and Acid Exposure

A higher swallowing rate was associated with a lower distal esophageal baseline impedance when analyzing all subjects ($P = 0.014$, Fig. 5A). Furthermore, maximal Barrett segment length in patients increased with lower baseline impedance ($P = 0.004$, Fig. 5B). The correlation between swallowing rate and esophageal baseline impedance was still present when tentatively excluding patients with columnar epithelium at the site of impedance measurement as explained in the legend for Figure 5 ($P = 0.007$). No correlation was present between baseline impedance and ACT (all $P > 0.3$).
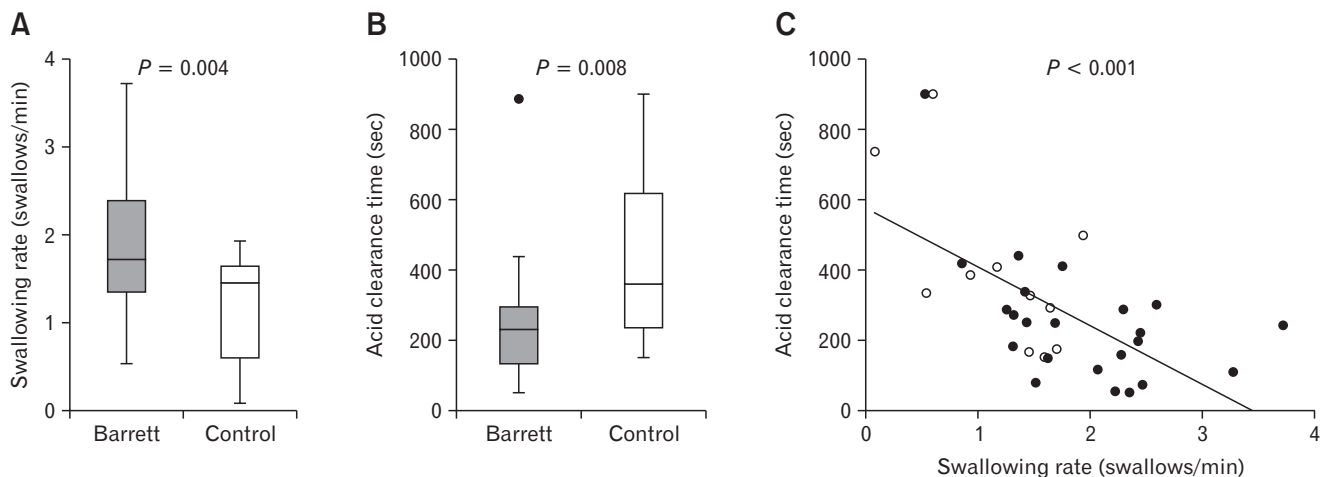
Figure 4. Swallowing rate and acid clearance time measured during the random swallowing trial. Compared with controls, patients with Barrett's esophagus swallowed more frequently (A) and had faster acid clearance (B). Furthermore, these 2 parameters were inversely correlated (C). In the correlation, closed circles indicate patients with Barrett's esophagus and open circles controls.

Figure 5. Associations between mucosal baseline impedance, swallowing rate, and Barrett segment length. Swallowing rate (A) and Barrett segment length (B) both increased with lower baseline impedance. In (A), tentatively excluding patients with columnar epithelium at 5 cm above the lower esophageal sphincter (LES), thus performing the analysis based on only data from squamous epithelium, did not change significance ($P = 0.007$). In (B), only data from columnar epithelium are shown. The number of subjects with data from only squamous epithelium at this measuring site did not allow analysis. In (A), closed circles indicate patients with Barrett's esophagus and open circles controls.

Discussion

To identify acid clearance mechanisms in BE we used the standardized esophageal acid clearance test and ambulatory pH-impedance testing. During random swallowing, patients swallowed 60% more often than controls resulting in a 46% shorter acid clearance. However, ambulatory acid exposure was still increased. The greater swallowing rate may thus be interpreted as an insufficient anti-reflux mechanism. In line with this, no association was observed between acid clearance using the standardized test and that using ambulatory pH.

The only published study we are aware of in a BE population using the acid clearance test supports our findings: with comparable conditions including random swallowing, Orr et al[25] found faster acid clearance and shorter swallow latency in patients with BE than in controls. To our knowledge however, we are the first to show an increased swallowing rate in patients with BE and its correlation with shorter acid clearance time. In our control subjects, supine as well as upright acid clearance times were in line with other studies using the acid clearance test[34,37] and random swallowing rate also similar to that previously reported.[43,44]

Contrary to our findings in patients with BE, others have found prolonged acid clearance using controlled swallowing in the acid clearance test. However, these findings were based on a total of only 16 patients with BE. All of these patients had either EE, previous surgery, strictures, or other complications along with BE.[22,27]

Moreover, 2 studies[45,46] showed similar and 1 even better motility in BE compared with patients with EE.[47] The latter finding specifically supports our own results, although insignificant, showing a 64% longer acid clearance time in patients with BE and EE than in BE without EE.

In relation to ambulatory pH measurements, a number of studies confirm our findings of greater acid exposure in patients with BE compared with controls.[15,47-49] Only one ambulatory study showed prolonged acid clearance time in BE. However, their controls were 12 and 17 years younger than patients which could possibly have confounded the results.[3,50] A positive correlation between acid clearance times obtained with the acid clearance test and ambulatory monitoring, which we could not show, has been shown by only 2 studies,[21,51] neither demonstrating this relation in patients with BE. As for our results indicating that HH presence shortens acid clearance, previous studies have shown prolonged acid clearance in HH patients.[23,24] However, these studies included HH patients without BE and therefore cannot be used for comparison.

As for the selection process, previous data did not allow a true sample size calculation and thus sample size was chosen based on experience. Despite the objective criteria used, bias in the selection process cannot be ruled out, eg, the included patients may not be representative of the whole patient population. Except for one missing control, the groups were equal to the preselected sample size. However, this sample size was somewhat reduced by dropouts, possibly due to the extensive protocol including 2 visits and multiple

intubations.

In relation to demographic cofactors, some heterogeneity within groups is inevitable. Considering whether the investigated BE population is representative overall, also taking into account sample size, other largely similar studies have included widely comparable populations.[25,52-55] Yet, the older age and higher BMI in patients compared with controls in the present study and patient heterogeneity such as eg, presence of EE could pose a problem. However, specific analyses on these subjects to detect possible confounding did not change results. Furthermore, neither tentative exclusion of patients with EE, higher BMI, nor older age changed results. The same was true when subjects intubated orally were excluded. Furthermore, esophageal function decreases with age[56] and greater BMI is associated with more severe reflux.[1] Thus if these factors should have affected the results, the differences shown between groups would only have been reduced.

Conclusively, we find little reason to assume that our findings including increased swallowing and shorter acid clearance in patients are due to confounding. However, considering the limitations present, the results will need verification in future studies. In terms of external validity, our findings can only solidly be generalized to patients with BE.

In regard to the protocol, the oral intubation in some subjects might affect the results due to an effect on salivation.[57] However, a similar proportion in both groups was intubated either way. Furthermore, the results were unaffected when adjusting for intubation way as a cofactor as well as when tentatively excluding subjects intubated orally. Rebound acid hypersecretion due to the PPI break is possible, but most likely not important within the 4-day break used.[58,59] We believe that blinding of subjects to the test liquid used should assure unbiased results in terms of, eg, swallowing rate and heartburn. As a result of our chosen protocol, the random swallowing test was only performed in the upright position. However, others have shown similar findings in the supine position.[25] Esophageal manometry could have characterized motility parameters and swallowing better, but unfortunately was inaccessible. Ambulatory pH-impedance measurement is another option to assess esophageal acid clearance, which in reality however only measures acid and bolus exposure times and not the volume of the acid bolus being cleared.[19,60] Due to the high prevalence of HH in BE[61] and common large-volume as well as repeated reflux episodes (re-reflux) in HH patients,[24] ambulatory clearance times may be artifactually prolonged.[18,62] Thus, an ambulatory pH measurement showing increased acid exposure in BE cannot per se be considered evidence that esophageal acid clearance function is impaired. It may merely represent repeated and/or large-volume reflux episodes.

As opposed to the ambulatory measurement, the acid clearance test uses standardized reflux volume and acidity, thus minimizing possible confounding.[18] This difference probably explains the lack of association between the clearance times in the ambulatory measurement and those in the acid clearance test. However, a type 2 error is also possible. Although experimental, we believe the acid clearance test has clinical relevance as a physiological assessment. Previous findings show that most reflux episodes in patients with BE do reach a height corresponding to the acid infusion channel 15 cm above the LES in our setup.[3] Furthermore, we demonstrated a correlation between the objective parameter of baseline impedance and the somewhat subjective swallowing rate. This indicates a pathophysiological base for the findings. No previous data exist to contradict our finding of increased swallowing rates in patients with BE, on the contrary, one previous study came to a similar conclusion.[25] Future studies should reproduce our findings with other methods such as catheter-based ambulatory manometry. However, the swallowing rate thus measured could be biased, since the higher acid exposure in patients with BE in the clinical setting would theoretically also increase swallowing rates to a greater extent than in controls.[44]

We found that patients with BE had lower distal esophageal baseline impedance, which correlated with a greater swallowing rate. This was also true when only data from controls and squamous mucosa in patients with BE were included. Previous studies have linked low esophageal baseline impedance and impaired mucosal integrity to heartburn and acid hypersensitivity.[63,64] Accordingly, one previous study using controls for comparison also found indications of hypersensitivity to acid infusion in BE.[55] Opposed to this, 3 studies demonstrated hyposensitivity to acid perfusion in BE, all however using patients with EE as the control group.[52,54,65] Since patients with EE show sensitization and more mucosal inflammation than BE,[66,67] hypersensitivity in EE relative to BE could likely explain the relative acid hyposensitivity in BE reported in these studies.[52,54,65] In this regard, our finding of more severe heartburn in patients with BE during the acid clearance test could indicate acid hypersensitivity. We believe that the lower baseline impedance indicates impaired mucosal integrity, which would allow faster ion (ie, acid) transfer across the cell membrane to reach acid receptors.[68] The resulting acid hypersensitivity could possibly explain the increased swallowing rate and faster acid clearance in patients with BE.

The paradoxical findings of concurrent longer ambulatory acid exposure time in patients with BE despite shorter acid clearance

time during the random acid clearance test need an explanation. Factors such as esophagogastric junction incompetence, presence of HH, and large-volume nightly reflux all increase the amount of gastroesophageal reflux,[1,25,61] most likely also in our patients with BE. We believe the increased swallowing rate is a physiologic compensatory mechanism to resist this reflux. However, since acid exposure in BE is still greater, the compensation seems to be insufficient, which could explain the above mentioned paradox. A clinical solution to the massive reflux that appears to be the problem could be anti-reflux surgery, which has been shown to prevent progression in BE.[69] Alongside that, standard medical treatment remains to be PPI guided by symptoms.[1]

Conclusively, we have proposed novel esophageal pathophysiology in BE such as more frequent swallowing and the association of this parameter to lower baseline impedance. In future research, it seems essential to confirm these findings and to gain further insight into the exact underlying sensory-mechanical interactions. The ambulatory pH-impedance measurement remains the state-of-the-art method for assessing the magnitude of reflux in gastroesophageal reflux disease including Barrett's esophagus.[70] However, our results suggest that this measurement in patients with BE will not always accurately represent esophageal clearance function. We propose that the greater acid exposure in BE may not be explained by an impaired acid clearance as such, but rather by a greater amount of reflux. Acid-evoked, protective reflexes seem to facilitate more frequent swallowing in an inadequate attempt to clear the refluxed acid. Despite this, increased volume and frequency of reflux episodes appear to tip the balance over time, leading to reflux-induced changes in the epithelium.

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